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CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

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CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

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AMERICAN ASSOCIATION OF CLINICAL CHEMISTS

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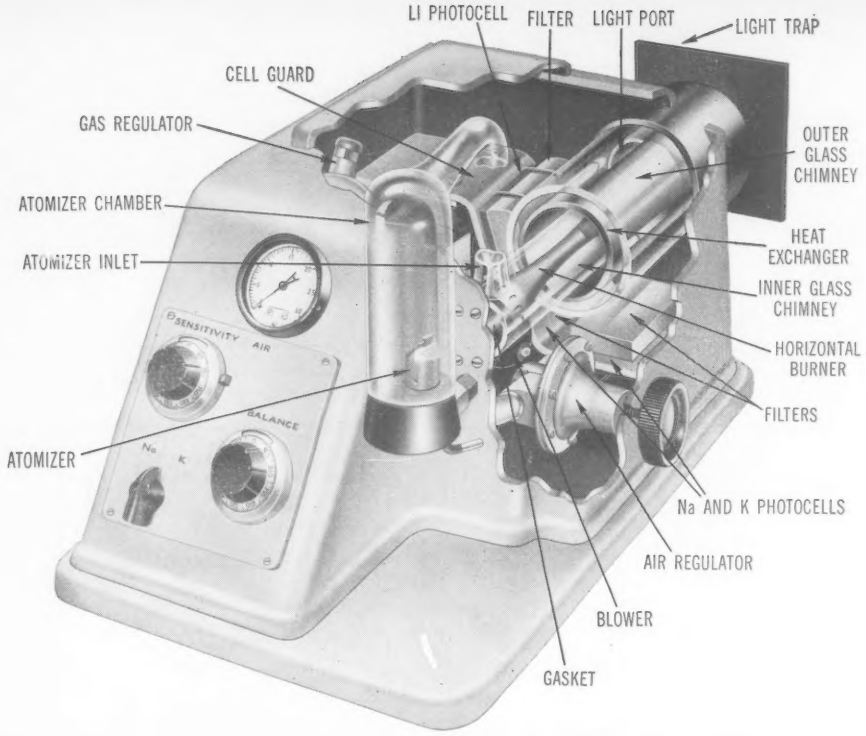
CONTENTS



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ABSTRACTS

from the

International Congress of Clinical Chemistry

New York, N. Y., September 9–14, 1956

Continuation of Abstracts of Papers Delivered Before the Congress

The role of magnesium in the body fluids. J. R. Elkinton (*Hospital of the University of Pennsylvania, Philadelphia, Pa.*)

Next to potassium, magnesium is the largest cationic component of intracellular fluid, yet relatively little is known of the role of this divalent ion in human disease. This is due primarily to methodologic difficulties and to the lack, until recently, of a radioactive isotope suitable for dilution studies. Many of the pharmacologic effects of magnesium are well-known, including its effect on respiration, on the nervous system, and on the cardiovascular system. Magnesium, like potassium, plays an essential part in many enzymatic reactions within body cells, and its distribution and transport must likewise be linked to metabolic processes. Factors governing magnesium absorption and excretion are not completely known; the relative roles of filtration, reabsorption, and tubular secretion in the kidney are being studied, and hormonal influences are under consideration. In some circumstances the magnesium content of the body appears to vary directly with that of potassium; in others, movements of magnesium are more closely allied to those of sodium.

Magnesium depletion has been observed classically in diabetic acidosis, and has been implicated in situations of inadequate intake, including postoperative parenteral alimentation and alcoholism; it recently has been produced experimentally in the normal human subject. Excesses of the ion are known to occur in renal disease. Its distribution is altered in hypothermic states, and possibly in thyroid disease. The pathophysiology of magnesium remains to be thoroughly explored and is a current challenge to the field of clinical chemistry.

Fermente des Glucose-6-phosphat-Dehydrogenasesystems im Blutserum. F. H. Bruns (*Institut für Physiologische Chemie der Medizinischen Akademie, Düsseldorf, Germany*)

Zwei Enzyme des Glucose-6-phosphat-Dehydrogenasesystems sind regelmässig in menschlichem Serum enthalten: 5-Phosphoriboisomerase (PRI) und Transketolase (TK). PRI katalysiert die Reaktion Ribose-5-phosphat (R-5-P) $\rightleftharpoons$ Ribulose-5-phosphat (Ru-5-P). Das Enzym erreicht in Trispuffer (pH-Optimum = 7,5) die grösste Aktivität und ist bei einer Konzentration von 0.01 m R-5-P

mit Substrat gesättigt (Km = $2{,}2 \cdot 10^{-3}$ Mol/R-5-P/Liter). PRI ist ein SH-Protein. Hemmung durch organische Quecksilberverbindungen (Salyrgan) hemmen die Enzymaktion, durch Cystein erfolgt Reaktivierung. Monojodacetat hemmt nicht. 1.0 ml normales menschliches Serum bildet je Std bei 37° und *p*H 7,5 durchschnittlich 3,5 Micromol Ru-5-P. Der Ketopentoseester kann mit Hilfe der Carbazol-Cystein-H_2SO_4 Reaktion von Dische leicht quantitativ erfasst werden. Erhöhte Enzymaktivität findet sich bisweilen bei Lymphosarkom, Lymphogranulomatose, chron. Nephritis und Lebercirrhose. Von 11 untersuchten Species besitzt Blutserum des Huhnes die höchste (33), dasjenige des Menschen die geringste Aktivität. Die Aktivität der roten Blutzellen ist beim Menschen rund 1000 mal höher als im Serum. Transketolase katalysiert das Gleichgewicht Ribose-5-phosphat + Ribulose-5-phosphat $\rightleftharpoons$ Sedoheptulose-7-phosphat (S-7-P) + D-Glycerinaldehyd-3-phosphat.

Inkubiert man Serum mit R-5-P, so bildet sich bei 37° mit einem Optimum bei pH 7,5–7,6 (Tris- oder Collidinpuffer) 0.025–0.1 Micromol S-7-P je Std und ml Serum. Da die PRI-Reaktion mehr als 30 mal schneller verläuft als die Transketolasewirkung, limitiert diese die Geschwindigkeit der Bildung von S-7-P, deren Konzentration sich leicht mit Hilfe der Orcin-Reaktion in den Trichloressigsäurefiltraten bestimmen lässt.

Suggestions for standardization in clinical chemistry. M. Guillot (*Faculté de Pharmacie, Université de Paris, Paris, France*).

At first, an attempt is made to give an adequate definition of true *clinical* methods in biochemistry. In any case these may be either (1) elaborate analytical technics allowing to make specific *dosage* of *one* definite pure chemical substance of physiologic or pathologic interest (ex: uric acid in urine by Ronchèse technic), or (2) half-specific technics giving only an *approximate valuation* of the true unknown amount of any *definite* substance (ex: pyruvic acid in blood), or even (3) analytical technics referring to a *group* of substances, quite analogous in their chemical properties (but eventually different in biologic importance or behaviour) (ex: 17-Keto-steroïds), and at last (4) every technic giving numerical *data* about any physical, chemical, or biologic *property*, notwithstanding lack of direct known connection of these data and any well-defined analytic characteristic of the biochemical sample thus concerned (ex: estimation of prothrombin by Quick test).

Indeed, in anyone of these four different cases, a *clinical* method is expected to possess two additional qualities: as it may be used as a *routine* method, it must (a) require but a *minute sample*, and (b) be performed in a *short time*.

As a consequence of the increasing development of research in biochemistry, there is a general tendency to extend the list of biochemical components to be studied, and on the other hand, the list of the different available methods of studying each one of them.

It will obviously become more and more necessary, in the future, to endeavor, at any time, a critical study of technics to be used in daily routine clinical work.

The main purpose of the present paper is to draw attention to the following points: (a) Necessity of an international standardization of methods. (b) necessity of reducing them in number (as far as clinical work is concerned); (c) necessity of referring to the classification above-mentioned: in any written report or printed paper, one would have to indicate the exact *rate* of any biochemical *named* component, only if in case 1; one would have to point at the *approximate* value of the *named* substance if in case 2; and at the numeral *estimation* of the *group* in case 3. Finally, in case 4, one would indicate the *rough data obtained in well-defined conditions, without mention of any named component.* Of course, in anyone of the four cases, each technic used would be fully specified (and must be, if possible, a well-known and standardized one).

The use of the aldolase activity test in the diagnosis of viral hepatitis. J. Hořejší, L. Mirčevová, R. Soušek, and K. Vaněček (*Central Biochemical Laboratory of the University Hospital, Charles' University, Prague, Czechoslovakia*)

The present state of the laboratory diagnosis of viral hepatitis is very unsatisfactory. Most of the routine tests are highly unspecific and the positive results appear rather late. Therefore we have studied the diagnostic value of the aldolase reaction, (the splitting of fructosediphosphate by sera of patients with hepatic lesions).

At first experiments have been made on animals treated with phosphorus and with atophane. Activity curves of aldolase in liver, kidney and muscle tissues have been determined. In another series of experiments the sera of 600 persons have been tested for aldolase activity. The results of our observations are as follows:

1. The aldolase activity test (AAT) is by no means specific for viral hepatitis. In all cases where liver has been damaged the AAT is positive.

2. The AAT is much more sensitive when compared with the results of other laboratory tests used in the diagnosis of hepatitis. The positivity is about 16 per cent higher than thymol turbidity reaction both in icteric and anicteric cases.

3. The positive results of AAT can be obtained in the very beginning of the illness, sometimes in the incubation period, before the infection becomes clinically manifest.

The possibility of using another substrate, hexosomonophosphate is discussed from the experimental as well as the clinical point of view.

A new rapid method for estimation of α and β lipoprotein cholesterol. J. Claes, A. M. Baerts, and J. V. Joossens (*Medical Departments and Central Laboratory of Clinical Chemistry, St Raphaël, University of Louvain, Belgium*)

About 0.2 ml of serum is submitted to electrophoresis on Whatman paper No. 1. Paper strips 6 cm. wide are used and the serum is applied in the usual way.

Using a horizontal electrophoresis apparatus and a Michaëlis Veronal buffer of *p*H 8.6 (M = 0.1) a satisfactory separation of lipoprotein is obtained in about 2 to 4 hours depending on the strength of current used. The strips are dried in the air and the lipoproteins located first by inspection under ultraviolet light or, if necessary, by cutting a small lateral track and dying it with Sudan Black or Oil Red. The strips are then cut in three parts of equal length containing respectively the α- and β-liproproteins and a paper blank. The method of Pearson, Stern, and McGavack for cholesterol estimation is now performed directly on the paper strip without extraction. The cellulose is dissolved in the mixture of acetic acid, paratoluenesulfonic acid, acetic anhydride and sulfuric acid (total volume 4.6 ml.) while the cholesterol is reacting in the usual way. After addition of the sulfuric acid the mixture must be stirred thoroughly to avoid charring of the paper. Photometric reading is done at 540 mμ, preferably in microcells with a 20 mm lightpath, against the blank. Sometimes centrifugation is necessary before the reading.

The percentage of cholesterol in the α- and β-fraction is calculated and the absolute values are obtained from the total cholesterol value. Since the percentage did not change by using larger or smaller amounts of serum the trailing in the α-lipoproteins could be excluded.

The method was compared with that of Langan, Durrum, and Jencks using extraction with chloroform-methanol and the reaction of Zlaktis, Zak, and Boyle for color development. Almost identical results were obtained.

Quantitative study of dye-protein relationship by scanning and elution methods in paper electrophoresis. J. V. Joossens and J. Claes (*Medical Departments and Central Laboratory of Clinical Chemistry, St Raphaël, University of Louvain, Belgium*)

The relation of dye and protein using naphthalene black (amidoschwarz) was studied by the wedge method of Rees and Laurence. Two commercially available manual scanners were used.

By plotting the area (proportional to the optical density, because the steps of the wedge were equally large) found on the scanning apparatus with 2 x 8 different wedges against protein concentration (each wedge bears at least 7 different protein concentrations), no such linear relationship could be obtained On double logarithmic paper a straight line was observed, suggesting an empiric relation of the form $D = aC^b$ (I) where a and b are constants, D is the optical density, and C is the protein concentration in arbitrary units. The most important factor in this equation is b, which is equal to the tangent of the line on double logarithmic paper. When b is equal to unity, Beer's law is obtained.

The constant b can also be calculated as a regression coefficient by arranging (I) into the logarithmic form, $\log D = b \log C + \log a$.

Statistically significant results were obtained with our scanners using Whatman No. 1 paper:

No oiling and white light: $b = 0.42$;

With oiling and white light: $b = 0.62$;

With oiling and an interference filter of 625 mμ (by substituting a Kipp galvanometer for the microamperometer of the apparatus used): $b = 0.75$.

The constant b is reproducible only when the dying technic is standardized for a linear relationship between elution and protein concentration. Heating to 105° before dying is not necessary. The constant b is also related to the type of photocell and to the geometry of the scanner. Each owner of an electrophoresis apparatus should determine the value of b in the conditions used by him. When it is found that constant b differs significantly from unity, a corrected scale may be constructed according to (I) for the galvanometer or microamperometer instead of the usual logarithmic one. By the use of such a corrected scale good correlation can be obtained between scanning and elution ($r = +0.9$ for 100 different serums).

Blood-protein-bound-iodine levels in resorcinol-induced thyroid gland hyperplasia. J. H. Webster (*Dept. of Pathology, University of Michigan, Ann Arbor, Mich.*)

The development of thyroid gland hyperplasia in humans and experimental animals following administration of resorcinol derivatives has been reported repeatedly. In order to maintain progressive hyperplasia it is necessary to maintain a constant level of the drug.

The initial change is one of epithelial hypertrophy; the cells lengthen, the ratio of cytoplasm to nucleus increases, the nuclei become more plump, are centrally placed, and show an increased prominence of the nucleoli. The acini enlarge, the colloid, which had been tightly pressed against the flat cuboidal epithelium, recedes from the epithelium. The colloid becomes more fluid, stains lighter, develops serrated margins and is gradually eliminated. The epithelial cells become tall columnar and show frequent mitotic figures.

These changes are secondary to an increased output of thyrotropic hormone, and it has been demonstrated that this hyperplasia cannot be maintained in the absence of the pituitary gland.

Correlation with the protein-bound-iodine levels has not been reported. Protein-bound iodine was determined on sera from heart blood of lightly anesthetized animals prior to sacrificing by air embolism. The base control level averaged 2.8 μg./100 ml. PBI was determined on separate groups of animals for a seven-week period. There was a transitory rise in PBI seen first at ten days and returning rapidly to normal levels by the fourteenth day. This return to normal levels was rapidly passed and the PBI then dropped to an undetectable level. The initial rise did not exceed a high of 5.2 μg./100 ml. and averaged 5.1 μg./100

ml. There was no subsequent detectable rise to the end of seven weeks and the levels were considered at or below 1 μg./100 ml.

The influence of uremia on the apparent cholesterol level in serum. A. M. Baerts, J. Claes, and J. V. Joossens (*Medical Departments and Central Laboratory of Clinical Chemistry, St Raphaël, University of Louvain, Belguim*)

During the course of a comparative study on cholesterol estimation in serum it became evident that the presence of uremia had a profound influence on the apparent results while using the methods of Pearson, Stern, and McGavack (1) and those of Zlatkis, Zak, and Boyle (2). In the absence of uremia, close agreement is obtained with (1) and the method of Sperry and Schoenheimer (3), used as a standard. The mean difference (1 minus 3) for 52 serums (varying between 80 and 763 mg./100 ml. cholesterol) was −2 mg./100 ml. (standard deviation of the difference 5.5 mg./100 ml. Method 2 was only satisfactory after deproteinisation and extraction by heat of the cholesterol with an acetone-ethanol mixture. The mean difference (2 minus 3) using the same serums was + 38 mg./100 ml. (S.D. 30.1 mg/100 ml.) without extraction. After extraction (32 serums) it amounted to +0.6 milligrams per cent (S.D. 5.7 milligrams per cent). With uremia there was a definite increase in apparent cholesterol with 1 and 2 as compared with 3. The error could amount to 96 milligrams per cent cholesterol for urea nitrogen values of about 200 milligrams per cent. 52 Serums from 17 patients at different levels of uremia were analyzed. The coefficient of correlation between the difference of cholesterol values (method 1 minus method 2) and urea nitrogen was +0.79 ($p < 0.01$). A regression line could be calculated and was found as $y = 0.68x - 33$. y represents the difference between both methods expressed in milligrams per cent cholesterol and x the values of urea nitrogen in milligrams per cent. Using this formula in the cases where urea nitrogen exceeded 50 milligrams per cent, it was possible to obtain values for serum cholesterol generally (59 times in 62) within ±20% of method 3. The exact nature of the substance reacting with method 1 and 2 is not known. It is neither urea, creatine, nor uric acid. It is soluble in ethanol-acetone and disappears, at least partially, by dialysis of the uremic serum against saline.

The influence of antibiotics, cortisone, meticorten, and soya lecithin on antibody production. C. A. Slanetz (*Columbia University, New York City.*)

Antibody production was studied in mice and rats fed natural foods stock diet supplemented with various antibiotics, cortisone, meticorten, and soya lecithin at levels corresponding to those used in human therapy. *Salmonella enteritidis* antigen was injected while the rats and mice were on the respective diets.

In one typical experiment the antibody titer of rats given cortisone was approximately one-half that of the controls. When soya lecithin (Granulestin) was fed, the titer was higher than that of the controls.

In mice it was found that cortisone alone interfered with antibody production

very markedly. When mice were injected with cortisone and fed diets containing antibiotics or antibiotics plus granulestin, the agglutinin titers of those animals were several times as great as those of the mice fed control diet.

Meticorten likewise depressed antibody production. A combination of penicillin and meticorten further lowered the antibody titer. Soya lecithin incorporated in the diet of mice and rats given diets supplemented with meticorten resulted in titers approaching that of the controls. A relationship was demonstrated between high antibody titer and resistance to live culture injections when vaccinated mice are used.

The urinary amino acids in relation to calculus disease. Mary G. McGeown (*Department of Medicine, Queen's University of Belfast, Ireland*)

It has long been recognized that considerably greater amounts of calcium salts are in solution in urine than would be possible in aqueous solution. The electrolytic effect of other salts, and the effect of urea and uric acid are not sufficient to account for this. It has often been postulated that the organic substances in urine play an important part in holding calcium salts in solution, in particular the urinary colloids, but the effect of these has not been clearly demonstrated. It has been shown that calcium chloride forms a crystalline compound with glycine, and that hydroxyapatite is more soluble in glycine and alanine solutions than in water. Since more than 6 Gm. of amino acids are excreted in the 24 hours, it seems possible that they may help to hold calcium in solution, possibly by the formation of soluble chelates. This has prompted us to look for abnormalities of amino acid excretion in calculus patients. Some of our patients excrete reduced amounts of all amino acids, while others show abnormal patterns of excretion of amino acids.

Experiments with rats on a stone-forming diet, some of which are being given supplements of amino acids, are being carried out. The results from the first batch of rats show that while some of the control animals developed stones, there were none formed in the rats that were given glutamic acid. This experiment is being repeated. Another experiment using mice is being carried out. The mice are given acetazoleamide because Harrison and Harrison (1955) have shown that it causes renal calcification or stone formation. In this experiment with mice there is also a significant difference between the control group and a group given glutamic acid.

The diagnosis of hyperparathyroidism. Mary G. McGeown (*Department of Medicine, Queen's University of Belfast, Ireland*).

The diagnosis of hyperparathyroidism has become of greater importance in recent years and it has become clear that it can exist without evidence of bone disease. Many cases of hyperparathyroidism form renal calculi, with or without renal calcinosis, and it has been suggested that up to 20 per cent of all cases of renal calculi may be due to this condition. In our stone clinic, all cases, except those with cystine or uric acid stones, are investigated to try to exclude hyperparathyroidism as an underlying condition. The investigations carried out include at

least four determinations of the serum calcium and phosphorus, the urinary excretion of calcium on a standard low calcium intake, the ratio of the renal clearance of phosphate to the glomerular filtration rate (which is a measure of the proportion of filtered phosphate unabsorbed by the renal tubules), the alkali reserve, the serum proteins, the serum alkaline phosphatase, and the blood urea. To date, 6 tumors and 4 cases of histologic hyperplasia have been found in 15 cases explored. It is hoped that further patients will have undergone exploration shortly. On analysis of the explored cases it has been found that hyperparathyroidism may be present although there are only minor changes in the serum calcium and phosphorus, and that hypercalcuria and hyperphosphaturia may be absent.

Serum and cerebrospinal fluid magnesium levels in conditions of altered electrolyte metabolism. A. A. Henly and R. A. Saunders (*Biochemistry Laboratory, Little Bromwich General Hospital, Birmingham, England*).

Calcium and magnesium levels were estimated in a series of patients with disorders of electrolyte metabolism, including gastroenteritis, poliomyelitis, and renal diseases. A preliminary appraisal of the results is given.

The role of a contaminant in thrombin in human plasmin assay systems. M. Siegel and E. E. Cliffton (*Sloan-Kettering Institute for Cancer Research and the Cornell University Medical College, New York, N.Y.*)

Two methods are in general use for assay of plasmin activity. One measures the proteolytic activity of the active enzyme on a casein substrate, the other measures the time required for the dissolution of a fibrin clot produced from a standard amount of bovine fibrinogen. Plasmin is prepared in vitro by the addition of streptokinase to a preparation of "human plasminogen"—at this time an inseparable mixture of proactivator and proenzyme (plasminogen). The literature contains many puzzling and contradictory reports on the high fibrinolytic coupled with the low fibrinogenolytic and caseinolytic activity of plasmin. Some authors have advanced a theory of enhancement of plasmin activity in the clotting process, others have explained the anomalous behavior on the basis of large amounts of bovine plasminogen in the fibrinogen substrate. Our measurements on the proteolytic activity of the plasminogen content of bovine fibrinogen showed that it could account for only $\frac{1}{25}$ of the enhanced activity. It has been our observation that anomalous behavior occurs only after clotting and we have therefore investigated the role that thrombin was playing in the assay systems. It soon became apparent that the quantity of fibrinolytic enhancement depended on the quantity of thrombin used for clotting. Fibrinolytic assay systems clotted with a small amount of thrombin required a long period for lysis; systems clotted with large amounts of thrombin lysed rapidly. That the clotting process itself was not required for enhancement was shown by proteolytic measurements. Proteolytic measurements of plasmin activity were greatly enhanced by the presence of thrombin, and this enhancement increased as the quantity of strepto-

kinase used for activation of the plasminogen was increased. In this respect the system behaved like a mixture of human plasminogen and bovine plasminogen. A highly purified thrombin preparation obtained from another laboratory and thrombin purified by starch electrophoresis in this laboratory failed to show enhancement. Therefore, enhancement is not associated with thrombin activity, but with some impurity in the thrombin. The impurity was separated from commercial bovine thrombin by starch electrophoresis and was shown to be similar enzymatically and physically to bovine plasminogen. The anomalous results in the literature in the light of this observation cease to be anomalous.

Spectrophotofluorometry: A new tool for analysis at the sub-microgram level. D. Duggan and B. Vasta (*Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Md.*)

Two commercial spectrophotofluorometers based upon the design of an experimental instrument developed at this Institute have recently become available. Each consists of the same essential components; a xenon arc source emitting a continuum throughout the region 200–800 mμ, an activating monochromator to isolate the exciting wavelength, a fluoroescence monochromator to analyze the fluorescence spectrum, and a suitable photomultiplier detector.

These instruments extend the utility of fluorescence measurement as an analytical tool in several direction. The availability of high intensity monochromatic activation at the precise optimum wavelength offers a distinct advantage over conventional fluorimeters whose source consists of a few widely separated lines of the mercury spectrum. The detection of fluorescence is not limited to the visible region, but is extended well into the ultraviolet. The ease with which both activation and fluorescence spectra are visualized obviates the necessity of prior knowledge of absorption or fluorescence wavelength data (as for the design of suitable primary and secondary filter systems), and provides a ready indication of the presence of interfering materials, light scattering, or the absorption of fluorescent light.

A point-by-point comparison of spectrophotofluorometry and conventional fluorimetry will be presented as well as spectral data illustrating typical applications of spectrophotofluorometry to the qualitative and quantitative analysis of biological constituents in tissues.

The nature of the Ehrlich benzaldehyde reaction on indolic compounds: I. Paper chromatographic, spectrophotometric, colorimetric, and spectrophotofluorometric measurements. H. Sprince, G. R. Rowley, D. Jameson, F. E. Manson, I. F. Bennett, and F. C. Dohan. (*U. S. Veterans Administration Hospital, Coatesville, Pa., and the Department of Psychiatry of the University of Pennsylvania, Philadelphia, Pa.*)

Current interest in psychopharmacology centers around certain newly synthesized 5-hydroxyindolic compounds (e.g. 5-hydroxytryptophan, serotonin, 5-hydroxyindoleacetic acid). This makes timely a study of the nature of the

Ehrlich benzaldehyde reagent (EBR) on these and related compounds to aid in the development of identification and assay procedures. (EBR is generally a 2% solution of *p*-dimethylaminobenzaldehyde in dilute HCl or dilute alcoholic HCl and gives a red-blue color with indoles.) Accordingly, the following compounds: (1) tryptophan, (2) 5-hydroxytryptophan, (3) tryptamine HCl, (4) 5-hydroxytryptamine creatinine sulfate (serotonin), (5) indoleacetic acid, (6) 5-hydroxyindoleacetic acid, (7) indole, (8) skatole, (9) indolepropionic acid, (10) indolebutyric acid, and (11) lysergic acid diethylamid tartrate were subjected to paper chromatographic, spectrophotometric, and colorimetric analysis after treatment with EBR in aqueous solution. Spectrophotofluorometric analysis was performed before and after treatment with EBR. The following data are presented in detail:

1. Paper chromatographic R_f values.
2. Spectrophotometric absorbance maxima.
3. Colorimetric limits of detectability and ranges wherein Beer's law is operative.
4. Spectrophotofluorometric activation and fluorescent maxima before and after treatment with EBR.

The nature of the Ehrlich benzaldehyde reaction on indolic compounds: II. Factors affecting the nature of the reaction. G. R. Rowley, H. Sprince, Dorothy Jameson, F. E. Manson, I. F. Bennett, and F. C. Dohan (*U. S. Veterans Administration Hospital, Coatesville, Pa., and the Department of Psychiatry of the University of Pennsylvania, Philadelphia, Pa.*)

The first paper of this series presented data on the paper chromatographic, spectrophotometric, colorimetric, and spectrophotofluorometric nature of the Ehrlich benzaldehyde reagent (EBR) on indolic compounds of psychopharmacologic interest. In this paper, the same compounds were studied with respect to factors affecting the EBR reaction with the individual compounds. Data will be presented on the effect of the following: (1) time, (2) temperature, (3) concentration of compound under test, (4) composition of EBR reagent (including variations in concentration of the *p*-dimethylaminobenzaldehyde reagent and acid used and the nature of the acid used), (5) nature of solvent system used, (6) *p*H, and (7) oxidizing and reducing reagents.

SCIENTIFIC EXHIBITS

Integrating Photoelectric Recorder for Paper Electrophoretic or Chromatographic Measurement. M. Eicher and J. Sendroy, Jr. (*Division of Chemistry, Naval Medical Research Institute, Bethesda, Md.*)

The exhibit shows a laboratory-assembled instrument which automatically scans, records, and integrates the area under curves corresponding quantitatively to the color distribution of the components in paper-strip electrophoresis or chromatography.

Spectrophotofluorometry: A new tool for analysis at the sub-microgram level. D. E. Duggan and S. Udenfriend (*Laboratory of Chemical Pharmacology National Heart Institute National Institutes of Health Bethesda 14, Md.*)

The exhibit consists of a demonstration of the operation of at least one of our three instruments, and an exhibit board illustrating the fundamental concepts of fluorimetry, comparing spectrophotofluorometry to conventional fluorimetry, and showing reproductions of graphic data illustrating typical applications to both qualitative and quantitative analysis.

The sweat test: For the diagnosis of cystic fibrosis of the pancreas. H. Shwachman, M. Stern, R. R. Dooley, and E. Higgins (*The Children's Medical Center, Boston, Mass.*)

The sweat test is the most reliable single test for the diagnosis of cystic fibrosis of the pancreas.

A simple and practical method of collecting eccrine sweat is shown in this exhibit. A sample is analyzed for the concentration of sodium and chloride. The results in a study involving over 1200 patients will be shown. The values of the sweat sodium and chloride concentration in healthy as well as sick infants and children with a variety of miscellaneous conditions is approximately 20 mEq./L. In 200 patients with cystic fibrosis of the pancreas the average value is 120 mEq./L. for sodium and chloride. No other condition was encountered in which the sweat electrolytes were elevated to the same degree as in patients with cystic fibrosis of the pancreas.

Prior to the development of this test the most reliable method of establishing the diagnosis of cystic fibrosis was based upon an examination of the duodenal fluid for pancreatic enzyme activity. In approximately 15 per cent of patients with cystic fibrosis of the pancreas the pancreatic trypsin test is positive at a time when the sweat electrolyte test shows evidence of this disease. We have previously demonstrated the progressive loss of pancreatic function in children who have the pulmonary manifestations of this disease. In these patients the sweat electrolyte abnormality is present from birth and serves as a better index of this generalized disease.

In our clinic the sweat test has replaced the duodenal intubation as a diagnostic procedure primarily because it is much simpler, more reliable, and less traumatic to the patient.

New Biuret Reagent for the Determination of Proteins in Cerebrospinal Fluid

Harold L. Rosenthal and Helen I. Cundiff

THE BIURET REACTION has become an important analytic procedure for rapid and accurate determinations of protein concentrations in biologic fluids. Since the initial reports of Riegler (1), Autenrieth and Mink (2), and Autenrieth (3), who showed that proteins react with alkaline copper solutions to yield a violet-blue color, many modifications have appeared to improve the stability of alkaline copper solutions and to define optimal conditions for the reaction. These studies have resulted in biuret procedures for serum protein that yield results equivalent to those obtained by micro-Kjeldahl analysis (4–9).

In applying the biuret procedure (10) to cerebrospinal fluid protein determinations, we observed that some undiluted fluids become sufficiently turbid to invalidate the analyses. Initial experiments indicated that the turbidity could be prevented by the addition of a chelating agent, disodium ethylenediamine tetraacetate (EDTA) to the biuret reagent. The results of this study and the development of a new copper reagent form the basis of this report.

MATERIALS AND METHODS

Biuret Reagent

The reagent described by Fister (10) was used during the initial phases of this study.

Micro-Kjeldahl Determinations

These were performed by the procedure of Pregl with selenium oxychloride as catalyst. The analyses were corrected for nonprotein nitrogen determined on trichloroacetic acid filtrates.

From the Division of Biochemistry, Department of Pathology, The Rochester General Hospital, Rochester, N. Y.

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Standard Protein[1]

Stock Solution: Human serum albumin (American Red Cross) is diluted with physiologic saline to contain approximately 50 mg. per ml. as determined by Kjeldahl analysis. Albumin standard solutions, preserved with chloroform or thymol, are stable at 4° for about 1 month. Solutions kept frozen at −20° are stable indefinitely.

Working Solution: Dilute 5 ml. albumin stock solution to 100 ml. with physiologic saline. This solution is stable for about 1 month in the refrigerator when preserved with chloroform. A slight turbidity may develop which does not interfere.

Analytic Procedure

Place 2 ml. saline, albumin working standard, and cerebrospinal fluid respectively in 3 test tubes. Add 3 ml. biuret reagent and mix by inversion. After 20–30 minutes at room temperature determine the absorbence in a suitable photoelectric colorimeter adjusted to 0 absorbence with the saline-biuret reagent blank.

The Klett-Summerson and Sheard Sanford Colorimeters with filters transmitting at 540 or 550 mμ have proved to be satisfactory in this laboratory.

EXPERIMENTAL

Effect of EDTA on Development of Turbidity

The inclusion of graded amounts of EDTA to saline-biuret blank solutions results in a linear decrease in absorbence until a constant minimal absorbence is obtained at concentrations of EDTA greater than 15 mg. per cuvette (Fig. 1). In the presence of cerebrospinal fluid, the decrease in absorbence parallels blank absorbence when the EDTA concentration is greater than 5 mg. per cuvette. However, in the absence of EDTA or at concentrations of EDTA lower than 5 mg. per cuvette, the absorbence of solutions containing cerebrospinal fluid is disproportionately high and does not parallel the absorbence of the blank solutions. In the absence of EDTA, many of these solutions appear visibly turbid. The optimal quantity of EDTA was, therefore, arbitrarily selected as 18 mg. per cuvette.

[1] Commercially available standardized human serum albumin (Pro-Sol) distributed by Standard Scientific Supply Corporation, New York, N. Y., has been found to be a satisfactory standard.

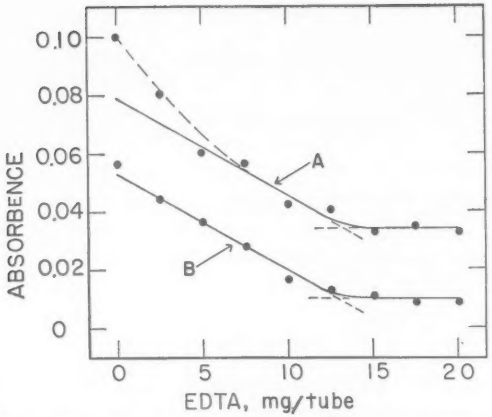

Fig. 1. The effect of disodium ethylenediamine tetraacetic acid (EDTA) on absorbence. Curve *A*, cerebrospinal fluid. Curve *B*, blank solutions.

Cause of Turbidity

The alkaline earth elements calcium and magnesium appeared to be the most likely substances present in body fluids to cause turbidity under the alkaline conditions of the biuret reaction.

In order to test this assumption more completely, known quantities of calcium were added to a series of albumin standards.[2] The protein solutions were then tested by the analytic procedure and the absorbences obtained with the biuret reagent (10) compared with that of the reagent containing an optimal amount of EDTA as determined above.

It is apparent that small amounts of calcium result in increased absorbence, which is effectively prevented by the addition of EDTA (Fig. 2).

Sensitivity of Reagents

Standard curves prepared with the reagent alone or containing optimal quantities of EDTA are shown in Fig. 3. It can be seen that the presence of EDTA does not alter the proportional development of color. Although EDTA decreases the sensitivity of the reaction, the range of concentration over which the response is linear is not appreciably altered. The range is sufficient to include the majority of protein concentrations usually encountered in cerebrospinal fluids.

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[2] Dilute albumin standards were found to be free of calcium.

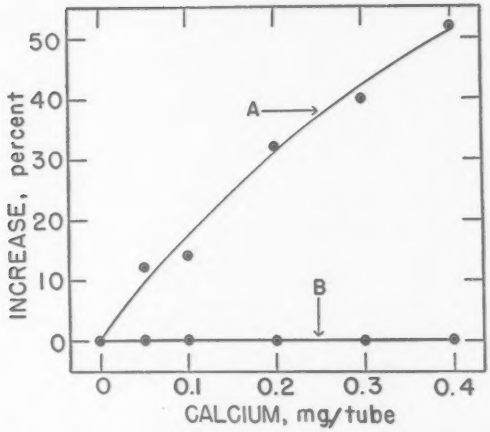

Fig. 2. The effect of calcium concentration on the biuret reaction. Curve *A*, biuret reagent alone. Curve *B*, biuret reagent containing the optimal amount of EDTA.

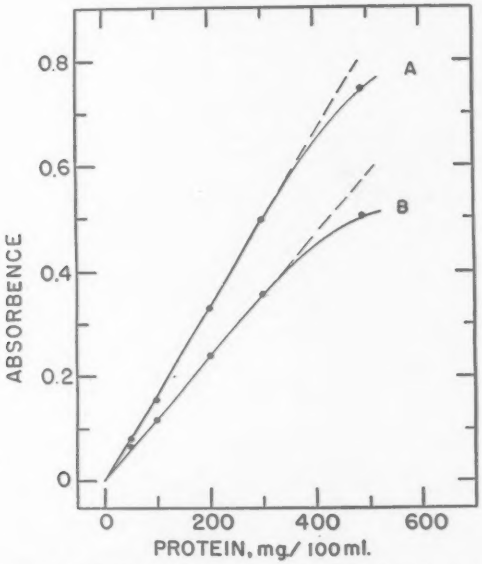

Fig. 3. Relationship between protein concentration and absorbence. Curve *A*, biuret reagent alone. Curve *B*, biuret reagent containing the optimal amount of EDTA.

Absorption Spectra

Absorption spectra of albumin solutions treated with biuret reagent alone or with biuret reagent containing an optimal amount of EDTA were obtained with a Beckman DU spectrophotometer. The shape of the spectra were similar with both reagents, exhibiting a broad absorption curve with peak absorbences at 550 mμ. Maximum absorbence, however, was depressed in the presence of EDTA as was to be expected from the decreased sensitivity of the reaction.

New Biuret Reagent

With the above information available, a new biuret reagent was prepared as follows:

Dissolve 1.50 Gm. Cu $SO_4 \cdot 5H_2O$ in about 500 ml. water. Add 6.0 Gm. disodium ethylenediamine tetracetate (EDTA) and 1.0 Gm. KI and dissolve. Add 300 ml. 2.50 *N* NaOH, mix well, and dilute to 1 L. The reagent is stable when stored at room temperature in polyethylene containers.

RESULTS

A series of protein determinations was carried out on cerebrospinal fluid with various biuret reagents and these were compared with values obtained by micro-Kjeldahl analysis. In order to obtain sufficient material for all comparative analyses, cerebrospinal fluids obtained by cisternal puncture at autopsy within 3 hours after death were used. These samples were preserved with chloroform and refrigerated until all analyses could be completed.

Table 1. Comparison of Protein Concentration in Cerebrospinal Fluids Obtained by Various Procedures

Sample	*Kjeldahl*	*Biuret reagents*		
		Fister (10)	*Fister (10) + added EDTA*	*New*
1	37	61	40	35
2	49	56	36	35
3	36	65	40	40
4	159	189	175	182
5	296	287	278	284
6	58	53	62	54
7	57	61	62	59
8	73	68	75	59
9	48	73	57	54
Average (mg./100 ml.)	90	101	92	89

The values obtained by the micro-Kjeldahl method and the new biuret reagent are in good agreement as shown in Table 1. On the other hand, it is evident that the original biuret reagent (10) yields significantly greater values than those obtained by either the micro-Kjeldahl or the new reagent. It is also apparent that the addition of EDTA to the original biuret reagent yields values comparable to those obtained by Kjeldahl analysis or by the new reagent.

DISCUSSION

The biuret reaction for the determination of proteins of biologic interest would appear to be the method of choice when all factors of accuracy and time are considered. However, the necessary precision and accuracy of the method can only be obtained by rigid control of conditions for the reaction, as is evident from the many modifications which have appeared in the literature. The present modification appears to be most useful when protein concentrations of less than 300 mg. per 100 ml. are to be determined. At such low protein concentrations a small degree of turbidity may yield values that are significantly too high. The use of the new EDTA reagent offers no real advantage for the determination of serum proteins since dilution of these materials prior to analysis decreases the calcium concentration to levels which do not seriously interfere. However, the lower blank absorbence obtained with the new reagent may be desirable. When applied to determinations of serum proteins the new reagent yields results comparable to Kjeldahl analysis.

Occasional observations have been made on the reduction of the copper salt of the reagent in the presence of biologic fluids, as has previously been observed by Weichselbaum (8). The reduction of copper, to yield a pale green-to-orange colloidal suspension, occurred only when the biuret reaction tubes had remained at room temperature for over 40 minutes. A second determination on the same material failed to result in reduction of the copper. On one occasion during the past 12 months, reduction of the reagent occurred with the standard albumin solution and again the reduction was not apparent until the solution had stood for 40–60 minutes. Since the reduction is not consistent or reproducible, we believe that the effect may be due to errors in the cleansing of glassware, pipets, etc., but we have not investigated the effect further.

SUMMARY

A new biuret reagent is described which contains disodium ethylen diamine tetraacetate as the copper stabilizing agent. The reagent effec-

tively prevents the formation of cloudiness in determinations of cerebrospinal fluid due, in part, to the precipitation of calcium in alkaline solution. Results obtained with the new reagent compare favorably with those obtained by Kjeldahl analysis.

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Quantitative Cephalin-Cholesterol Flocculation Test for Liver Function

Andre C. Kibrick

With the technical assistance of *Anthony Gargano* and *David Brooks*

THE USEFULNESS of the cephalin-cholesterol flocculation test for liver function (1) has been increased considerably by the quantitative modifications proposed by Saifer (2) and by the present workers (3). The method of Saifer has not been adopted in many laboratories since it involves an additional determination of cholesterol in the precipitates. Our method has not been adopted to any great extent although it involves merely a measurement of absorption in the centrifuged supernatant fluid with a photoelectric colorimeter. We reported the range of normal and abnormal values observed with two photoelectric instruments. It has been suggested that the values should be reported in percentage of the absorption of the control tube with saline. This would not be affected by the type of colorimeter that is used nor by the variations in the reagent. It was desirable to test the method performed in this way on the photoelectric instruments available in a new laboratory. The present report on the results found in 376 patients confirms our earlier evaluation of the quantitative cephalin-cholesterol flocculation test (3) and shows the range of normal and abnormal values that would be encountered with any photoelectric instrument.[1]

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[1] The present study was made primarily with Bausch & Lomb and Coleman Junior spectrophotometers. The latter is well known to yield low values of absorption in turbidity measurements. Some of the values were checked in the present study with a Lumitron photoelectric colorimeter. In the original study (3) the Klett-Summerson and Evelyn photoelectric colorimeters were used. From our experience with these instruments we believe that the range of normal and abnormal values reported in the present study would be encountered with any photoelectric instrument.

MATERIAL AND METHODS

The cephalin-cholesterol flocculation test was performed as described previously with a reagent prepared from a commercial antigen.[2] A 0.2-ml. portion of fresh serum from fasting patients was mixed with 4 ml. of normal saline in 15-ml. centrifuge tubes, and 1 ml. of the reagent was added. The mixture was mixed well, stoppered with a cork, and allowed to stand in the dark at room temperature for 24 hours. A control tube in which 0.2 ml. of normal saline was substituted for serum was included in each series of tests. After standing, the tubes were evaluated qualitatively from 0 to 4+ in the usual manner and were then centrifuged for 15 minutes at 1200 rpm. The supernatant fluid was poured into 14–15 mm. cuvettes and the absorbence was measured against water at 650–660 mμ in a Bausch & Lomb and in a Coleman Junior spectrophotometer. The absorbency values were then calculated in terms of percentage of the control tube by the following equation:

$$\frac{\text{optical density control} - \text{optical density unknown}}{\text{optical density control}} \times 100 = \text{percentage}$$

RESULTS

In Fig. 1 the qualitative results are plotted against the quantitative results calculated in this way. It is apparent that the scattered points may be described rather well by a smooth S-shaped curve, and that the degree of scattering may be ascribed to the inaccuracy of the qualitative evaluation of the test. The lower bend of the curve, which encompasses all but four of the negative qualitative values, is at 40 per cent. As a first approximation 39 per cent was assumed to be the upper limit of normal. The results were then evaluated on this basis by comparing them with the clinical condition of the patients and with the results of other tests for liver function.

The presence or absence of liver disease was established from the clinical condition of the patients and from the results of other tests for liver function. Thymol turbidity was performed in a spectrophotometer according to the method of Shank and Hoagland (4), and a value of 5 in their units was taken as the upper limit of normal. Serum albumin and globulin were estimated with the Kingsley biuret reagent after fractionation with sodium sulfate according to the original method, and a value

[2] Difco Laboratories, Detroit.

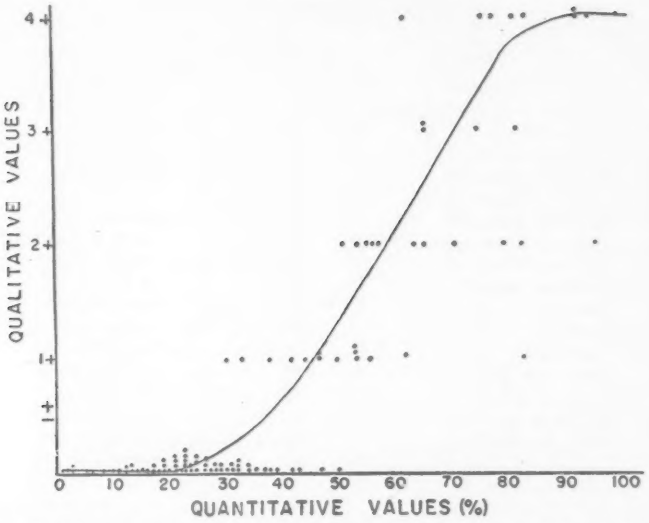

Fig. 1. Scattergram comparing qualitative and quantitative values of cephalin-cholesterol flocculation test.

of at least 1.5 to 1 for the albumin-globulin ratio was considered normal. Bilirubin was determined by the method of Malloy and Evelyn (5), in which 0.8 mg. per cent was considered the limit of normal. Cholesterol esters were determined by the method of Zlatkis *et al.* (6) with normal values at least 70 per cent. Alkaline phosphatase was measured in King-Armstrong units with a range of 4 to 12 as normal for adults. Bromsulfalein retention was determined in a photoelectric colorimeter after intravenous injection of 5 and 2 mg. per Kg. A retention of less than 5 per cent in 45 minutes for the larger dose and of less than 2 per cent in 60 minutes for the smaller dose was considered normal.

Representative results are shown in Tables 1 and 2 on a consecutive series of 80 serums. Included are the diagnoses and other pertinent chemical findings. It is evident from Table 1 that 29 of 35 samples with values of 40 per cent or more show evidence of liver disease. It is seen in Table 2 that only 5 of 45 serums with values below 40 per cent have any indication of liver dysfunction. The values of the cephalin-cholesterol flocculation test in 4 of the 5 discrepant samples are 38, 37, 34, and 33, which are not far removed from positive values. Other results on 171 patients with values below 40 per cent and 125 patients with values above 40 per cent are similarly consistent with the findings of other liver function tests

Table 1. Sera with Positive Values[a]

Patient	Diagnosis	Cephalin-cholesterol		Other
		Quantitative	Qualitative	
1	Cirrhosis of liver–alcoholism	100	4+	E 60, Th 10.8
2	Cholecystitis	96	2+	Th 11.2
3	Cirrhosis of liver	92	4+	A 2.8, G 4.7
4	Infectious hepatitis	92	4+	Th 7.2
4[b]	Infectious hepatitis	83	4+	E 50
5	Infectious hepatitis	83	1+	E 52
6	Cirrhosis of liver–alcoholism	82	2+	E 67, A 2.8, G 3.4
7	Chronic alcoholism	82	3+	A 3.3, G 2.5
8	Infectious hepatitis	81	4+	E 56
9	Infectious hepatitis	80	2+	A 3.2, G 4.1
10	Carcinoma of stomach	79	4+	A 2.5, G 3.2
4[c]	Cirrhosis of liver	75	4+	G 2.3
11	Cirrhosis of liver	73	3+	B 12.5
12	Cirrhosis of liver	71	2+	A 4.3, G 3.7
13	Infectious hepatitis	65	3+	B 2.0
8[b]	Infectious hepatitis	65	3+	B 3.4
14	Cirrhosis of liver	63	2+	Th 6.9
15	Cirrhosis–diabetes	62	1+	
16	Alcoholism–pneumonia	58	2+	
9[b]	Infectious hepatitis	57	2+	
17	Cerebellar tumor	56	1+	
18	Infectious hepatitis	55	2+	B 2.2
19	Infectious hepatitis	54	2+	A 4.4, G 3.3
20	Infectious hepatitis	53	1+	Th 12.0
21	Peptic ulcer–alcoholism	53	1+	B 0.4
18[b]	Infectious hepatitis	51	2+	A 3.8, G 4.1
22	Myocardial insufficiency	50	1+	B 1.9
23	Gastritis–alcoholism	50	0	E 24
24	Gastric resection–alcoholism	47	1+	
25	Gastric malignancy	47	0	E 69
26	Prolapse of gastric mucosa–alcoholism	44	1+	A 3.5, G 3.0, AP 31
27	Pilonidal cyst	43	±	A 4.8, G 3.0
9[c]	Infectious hepatitis	42	1+	B 1.1
28	Bronchogenic cancer–alcoholism	42	0	B 0.5
29	Infectious hepatitis	42	0	B 3.1

[a] Abbreviations: Th = units thymol turbidity; A = albumin, Gm. %; G = globulin, Gm. %; B = bilirubin, mg. %; E = % cholesterol esters; AP = alkaline phosphatase, K-A units; BSP = bromsulfalein.

[b] Second test about 10 days after first test.

[c] Third test about 10 days after second test.

and with the clinical condition of the patients. Evidence of liver dysfunction was obtained in only 15 of the 171 patients with low values of cephalin-cholesterol flocculation, and only 10 of the 125 patients with high values failed to show other evidence of liver damage.

Table 2. SERA WITH NEGATIVE VALUES[a]

Patient	Diagnosis	Cephalin-cholesterol		Other
		Quantatitive	Qualitative	
1	Cirrhosis of liver	38	1+	B 1.4
2	Diverticulosis of colon	38	0	
3	Anxiety reaction	37	0	Th 6.9
4	Cellulitis of head and face	35	0	
1[b]	Cirrhosis of liver	34	0	
5	Rheumatic heart disease	33	1+	Th 7.5, B 5.4
6	Pneumonia	32	0	
7	Peripheral neuritis	32	0	B 0.6
8	Cardiospasm	32	0	
9	Recovered infectious hepatitis	31	0	
10	Pneumonia	31	0	E 69
11	Rheumatic heart disease	29	1+	B 0.8
12	Alcoholic intoxication	29	0	BSP neg.
13	Ulcerative colitis	29	0	
14	Conversion reaction	28	0	
15	Psychoneurotic heart disease	28	0	
16	Gastroenteritis	27	0	A 4.3, G 2.8
17	Cervical cord disease	27	0	
18	Ear infection	27	0	
19	Chronic schizophrenia	26	0	B 0.8
20	Duodenal ulcer	25	0	
21	Bleeding marginal ulcer	25	0	
22	Basal cell carcinoma of leg	25	0	
23	Senile emphysema	24	0	B 0.8
24	Tuberculosis	24	0	B 0.7
25	Chronic bronchitis	23	0	B 0.9
26	Lymphosarcoma	23	0	
27	Neoplasm of the G.I. tract	23	0	B 1.4
28	Schistosomiasis	23	0	
29	Duodenal ulcer	22	0	
30	Cellulitis of leg	21	0	
31	Chronic cholecystitis	21	0	Th 3.0
32	Pilonidal cyst	19	0	
33	Cestodiasis	19	0	
34	Biliary tract neoplasm	18	0	B 0.8
35	Duodenal ulcer	17	0	
36	Acute gastroenteritis	17	0	
37	Chronic pancreatitis	16	0	
38	Angioneurotic edema	13	0	B 0.6
39	Fracture of left ankle	13	0	
40	Chronic duodenal ulcer	12	0	
41	Cellulitis of leg	3	0	
42	Acute pancreatitis	3	0	Th 2.4
43	Chronic pancreatitis	2	0	B 0.8
44	Carcinoma of neck	1	0	

[a]Abbreviations: Th = units thymol turbidity; A = albumin, Gm. %; G = globulin, Gm %; B = bilirubin, mg. %; E = % cholesterol esters; AP = alkaline phosphatase, K-A units; BSP = bromsulfalein.

[b] Second test approximately 10 days after first test.

This study substantiates our earlier evaluation of the quantitative cephalin-cholesterol flocculation test that it has "a sensitivity that compares favorably with the most sensitive tests for liver function" (3).

SUMMARY

The quantitative cephalin-cholesterol flocculation test of the author has been modified to give values in percentage of the absorption of the control tube with saline. This simple test which involves only a measurement of absorption in the centrifuged supernatant fluid may be used with any photoelectric colorimeter. Data are presented to show that in 29 of 35 serums giving values above 40 per cent the patients have other evidence of liver disease, while in only 5 out of 45 serums giving values below 40 per cent, the patients have possible liver damage. The same consistency in the results on 296 other patients is reported.

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Plasma Tributyrinase in the Neonatal Period

Including a Micromodification of a Titrimetric Method

Max M. Friedman and Robert L. Shriro

THE PRESENT STUDY was suggested by the lack of data on plasma tributyrinase (lipase) activity in the neonatal period. Titrimetric methods are not practical for small amounts of blood and it was found necessary to adapt the method of Goldstein *et al.* (1) to an electrometric procedure. Such a modification reduced to $\frac{1}{20}$ the amount of plasma and reagents needed in the titrimetric method. Troescher and Norris (2) determined blood esterase electrometrically by the addition of oxalated blood to a 0.15 M phosphate buffer at *p*H 8.9 with ethyl butyrate as substrate. It has since been shown that tributyrin substrate results in appreciably higher enzymatic activity (3) than does ethyl butyrate. The preparation of our substrate was identical with that described in *Standard Methods of Clinical Chemistry* (4) with the omission of sodium choleate (1). The modified electrometric procedure reported here has been used in our laboratory for routine clinical determinations of tributyrinase and found satisfactory.

METHOD

Blood specimens were obtained in the neonatal period at varying times between the first and sixth days of life. The heel was punctured with the tip of a Bard-Parker blade and the blood collected into capillary tubes coated with heparin. The dry end of the tube was gently

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warmed and sealed with red sealing wax. After centrifugation the hematocrit may be estimated, the plasma column cut off with a file, and the plasma transferred into small test tubes.

The buffered substrate containing calcium acetate, sodium barbiturate, Tween 20, Methocel, and tributyrin was prepared as previously described (4) and adjusted to *p*H 8.55. The *p*H of the substrate was found to shift to the acid side even under refrigeration, and it was necessary to make daily adjustments to *p*H 8.55 with dilute alkali. Although when stored in a deep-freezer the *p*H shift of the substrate proceeded at a slower rate, in no instance was the substrate used for longer than 1 week.

To 1.0 ml. of substrate warmed at 37° was added 0.05 ml. plasma and the test tube replaced immediately in a 37° water bath. After exactly 59 minutes the sample was removed from the bath and to it was added 3.0 ml. distilled water kept at 25°. The tube was then placed in a 25° water bath for exactly 1 minute and the contents transferred to a beaker of the Beckman Model G *p*H meter. A reading was taken within 30–45 seconds thus keeping the time error to a minimum. The activity was recorded in ΔpH units as the difference between the control and incubated specimens. The control reading was determined by dilution of 1.0 ml. substrate with 3.0 ml. H_2O, brought to 25°, and the *p*H read immediately after the addition of 0.05 ml. plasma. It was not necessary to incubate the control since no significant nonenzymatic hydrolysis of the substrate could be detected during the 1 hour incubation period. It was, however, necessary to add plasma to the control since the decrease of the substrate *p*H with plasma varied from 0.05 to 0.12 units. Duplicates by the electrometric method checked to within ± 3 per cent.

The error due to variations in the 60-minute incubation period should not exceed 1 minute. Attempts to stop the enzyme action by heating or with organic solvents introduced greater errors. Placing the specimen for 60 seconds in a boiling water bath destroyed the enzyme action but did not give reproducible results. Addition of organic solvents to precipitate the proteins resulted in instability of the glass electrode and gave erratic *p*H readings.

RESULTS AND DISCUSSION

We could not carry out a precise comparison of this electrometric method with the macrotitrimetric method (4) by determinating the same sample with both procedures. In our experience the titration of a buffered and turbid solution in an alcohol-ether medium did not give reproducible end-points with phenolphthalein. It was likewise impractical

to titrate to a potentiometric end-point since the glass electrode was unstable in a 70% alcohol-ether medium. A comparison of the two methods for the purpose of translating electrometric data to titrimetric units was, however, carried out in the following manner.

Butyric acid liberated in the hydrolysis of tributyrin by tributyrinase is back titrated with standard alkali in the titrimetric method. To simulate experimental conditions we started with 20 ml. substrate at *p*H 8.55 (without addition of alcohol-ether) and added increasing amounts of 0.1N butyric acid, determining the *p*H electrometrically after each increment of acid. Back titration with 0.1N NaOH from the lower *p*H gave a similar curve. This established the relationship between Δ*p*H and titrimetric units. Since both procedures require 1 part of plasma to 20 parts substrate, and since the plasma contributes to some extent to the total buffer capacity, the above titration was further adjusted by preparing a series of substrate–butyric acid mixtures and the *p*H read before and immediately after the addition of the requisite amount of plasma. The correlation between the titration units of the titrimetric method and the Δ*p*H units of the electrometric method is shown in Table 1. Plotted on coordinate axes the curves assume sigmoid forms, as would be expected in the titration of a buffer. The last column of Table 1, showing the titration with plasma, is the one recommended for translation of titrimetric to electrometric units.

Table 1. Comparison of Titrimetric and Electrometric Units Obtained from Titrations with and without the Addition of Plasma

Titration units	Δ*p*H *units without plasma*	Δ*p*H *units with plasma*
0.20	0.22	0.20
0.30	0.32	0.29
0.40	0.40	0.36
0.50	0.47	0.42
0.60	0.54	0.48
0.70	0.60	0.53
0.80	0.68	0.58
0.90	0.73	0.63
1.00	0.78	0.68
1.10	0.83	0.72
1.20	0.88	0.76
1.30	0.97	0.85
1.40	1.05	0.96
1.50	1.15	1.07
1.60	1.27	1.18
1.70	1.39	1.29
1.80	1.52	1.42

Table 2. SUMMARY OF PLASMA TRIBUTYRINASE LEVELS IN THE FIRST SIX DAYS OF THE NEONATAL PERIOD

Values recorded in ΔpH units

	Adults[a]	*Days after birth*		
		1–2	3–4	5–6
Determinations	100	67	44	25
Subjects	100	62	40	23
Total range	0.56–1.19	0.23–1.33	0.39–1.32	0.33–1.22
Range of 65% of results	0.68–0.96	0.54–0.82	0.54–0.76	0.63–0.88
Average	0.76	0.68	0.65	0.73
Standard Deviation[b]	±0.13[c]	±0.20	±0.17	±0.19

[a] Calculated from reference (4).

[b] Standard Deviation = $\sqrt{\Sigma \frac{d^2}{n-1}}$ (where d = average deviation, n = number of determinations).

[c] Estimated.

Table 3. PLASMA TRIBUTYRINASE LEVELS IN THE FIRST SIX DAYS OF THE NEONATAL PERIOD

Values recorded in ΔpH units

Subject	*Days after birth*					
	1	2	3	4	5	6
Za	0.70	0.69			0.70	
Bi	0.57	0.57			0.75	
Ta	0.58		0.70		0.64	
Wr	0.51		0.44			0.51
Wn	0.62		0.54			0.70
Ka	0.53	0.60			0.73	
Gl	0.60		0.60	0.58		
Sp	0.77		0.76	0.96		
Fe	0.82			1.00	1.02	0.98
Gu	1.25	1.16		1.32	1.22	
Ri	0.69	0.60				0.71
Va	0.69	0.60				0.76
Zu	0.73		0.89		0.88	
St	0.65		0.59		0.55	
Do	0.73		0.59		0.63	

The results obtained in the neonatal period were tabulated into three groups: those enzyme levels in the first and second days, the third and fourth days, and the fifth and sixth days. This grouping is arbitrary for the purpose of ascertaining whether any significant differences could be detected in the first days of life. In all, 136 determinations were carried out on 68 newborns. It may be seen from Table 2 that the average and the total range of values in the three neonatal periods vary only slightly.

The serum tributyrinase levels of 100 blood donors reported in *Standard Methods of Clinical Chemistry* may be used as the adult levels for a comparison with the neonatal period. These figures were given (4) as 1.20 ± 0.20 titrimetric units, with a total range of 0.76 to 1.61, and a range of 65 per cent of the values as 1.01 to 1.40 units. Translated into electrometric units of this method from Table 1 they would show an average ΔpH of 0.76 ± 0.13, a total range of 0.56 to 1.19, and a range of 65 per cent of the results as 0.68 to 0.96. These calculated data for adults are included in Table 2.

Table 3 contains the data obtained when three or more determinations were carried out on the same subject within the first 6-day period. With few exceptions, the enzyme levels are relatively constant in the same subject.

Figure 1 shows the frequency distribution of all the results obtained in the neonatal period. Of these, 80 per cent are in the range of 0.40 to 0.80 electrometric units, and 89 per cent between 0.40 to 1.00 units.

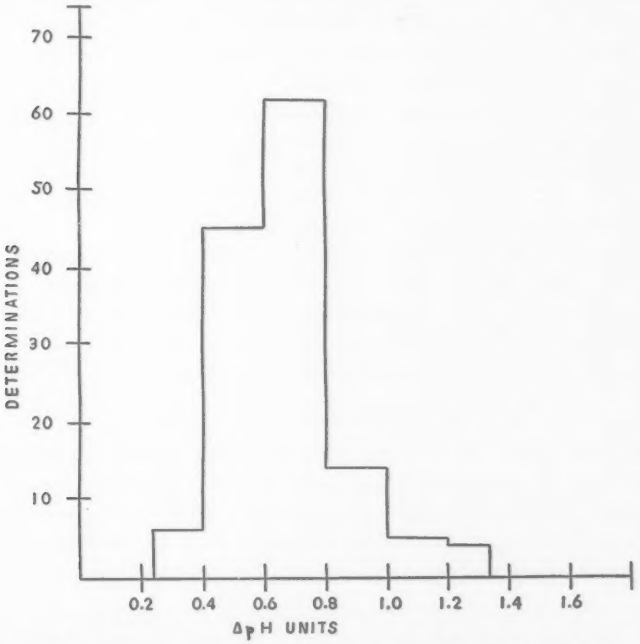

Fig. 1. Frequency distribution of plasma tributyrinase in the newborn.

SUMMARY

A method is described for the microestimation of tributyrinase activity in plasma that requires only 0.1 ml. of sample. This is an electrometric procedure adapted from a titrimetric method.

A comparison curve is established which relates titration units to electrometric units.

Data are presented for the plasma tributyrinase levels in the first 6 days of neonatal period. No significant daily variations could be detected.

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Glutamine and Glutaminase in Blood

Frank L. Iber and Joseph Bruton

RECENTLY THE NEED of a method for the determination of glutamine and glutaminase in the blood and urine of humans led to the evaluation and adaptation of a single system for both. Methods of glutamine assay available depend on the determination of the ammonia liberated by mild hydrolysis with dilute hydrochloric, sulfuric, or trichloroacetic acid, or alkali, heat at neutral *p*H, or the action of the specific enzyme, glutaminase (1). Other methods measure the decreased reactivity with nitrous acid or ninhydrin that occurs when glutamine undergoes hydrolysis and condensation to ammonium pyrrolidone carboxylate. Krebs (2) demonstrated that the cortex of kidneys of sheep, guinea pig, pig, and rabbit contained an enzyme, glutaminase, which hydrolyzes glutamine to ammonia and glutamic acid. Archibald (3) has described the preparation and standardization of kidney glutaminase in a form which makes the enzyme readily applicable for microestimation of glutamine in blood and other biologic materials. Seegmiller, Schwartz, and Davidson (4) have described a glutaminase preparation of bacterial origin. It has been observed in rats that renal glutaminase activity increases fourfold during adaptation to ammonium chloride acidosis (5). This led us to choose rats previously treated with NH_4Cl as the source of glutaminase preparation.

PREPARATION OF GLUTAMINASE EMULSION

Adult rats (250–300 Gm.) are made acidotic by giving them as drinking water a 2% NH_4Cl solution for 7 days. At the end of this period the animals are sacrificed by a blow on the head. The kidneys are removed and homogenized at 5° in a Waring Blendor in sufficient 0.9% saline to give a final concentration of 5 per cent. The homogenate is treated with

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5 ml. of 0.12M sodium cyanide and frozen for future use. This enzyme preparation is used for glutamine assay in blood.

ASSAY OF GLUTAMINASE ACTIVITY

Reagents and Equipment

1. 0.2M 2-amino-2-(hydroxymethyl)-1,3-propanediol
2. 0.6M Na_2HPO_4
3. 0.12M NaCN
4. 50% $HClO_4$
5. 0.2M glutamine solution
6. NH_3-free water
7. 2M H_3PO_4
8. 5-ml. Pyrex tubes with glass stoppers
9. Incubator set at 38°

Procedure

This procedure is that of Rector, Seldin, and Copenhaver (5) with modifications. To two 5-ml. Pyrex test tubes equipped with glass stoppers, add to each 0.30 ml. of 2-amino-2-(hydroxymethyl)-1,3-propanediol, 0.40 ml. of Na_2HPO_4 solution, and 0.15 ml. of NaCN solution. The *p*H of this mixture is adjusted to 7.5 with 2M H_3PO_4. Add to one tube 1 ml. of serum, plasma, or whole blood, 0.20 ml. of glutamine solution, and 0.20 ml. of NH_3-free H_2O. Add 1 ml. of serum, plasma, or whole blood and 0.40 ml. of NH_3-free water to the second tube. The tube contents are mixed well and incubated for 4 hours at 38°. After incubation add 0.15 ml. of $HClO_4$ to each tube, centrifuge, and take aliquots for NH_3 analysis. Glutaminase activity is therefore a function of the NH_3 liberated from the glutamine solution corrected for free NH_3 in the blank, and expressed as μM of NH_3 liberated per 100 ml. of plasma.

The glutaminase activity of the enzyme preparation is measured as above using 1.00 ml. of homogenate preparation in the place of blood and incubating the tubes for 1 hour at 38°. An aliquot is removed for NH_3 analysis. An aliquot of the homogenate preparation is dried at 100° in a tared tube and weighed. The glutaminase activity is expressed as μM of NH_3 per 100 mg. of dry tissue per hour.

ASSAY OF GLUTAMINE IN BLOOD

Reagents and Equipment

1. Same as those for glutaminase activity.
2. Glutamine standards (1, 2, and 3 μM/ml.).

Procedure

To each of two 5-ml. Pyrex test tubes equipped with glass stopper add 0.30 ml. of 2-amino-2-(hydroxymethyl)-1,3-propanediol, 0.40 ml. of Na_2HPO_4 solution, and 0.20 ml. of NaCN solution. Adjust the *p*H of this mixture to 7.5 with 2M H_3PO_4. Add 1 ml. of plasma, serum, or whole blood to the two tubes. Add 0.50 ml. of kidney homogenate to the sample tube, and 0.50 ml. of NH_3-free water to the blank tube. The contents of the tubes are mixed and incubated for 4 hours at 38°. An aliquot is removed for NH_3 analysis. An additional blank is prepared containing 1 ml. of H_2O and 0.5 ml. of kidney homogenate. This blank is to correct for any additional NH_3 arising from the homogenate during incubation. Glutamine standards of 1, 2, and 3 μM/ml. are analyzed with each run.

NH_3 ANALYSIS

Reagents and Equipment

1. Hydrochloric acid, stock solution: Standardized 0.100N HCl. Working sample: Dilute stock standard 1:20 to give 0.0050N HCl.
2. Barium hydroxide: From a saturated solution of $Ba(OH)_2$ prepare a working standard. Dilute stock 1:20 for approximately 0.03N $Ba(OH)_2$.
3. Microtitration table with mounted Scholander buret (7).
4. Beckman *p*H meter with standard probe electrodes.
5. Conway's microdiffusion cells.
6. Saturated K_2CO_3.

Procedure

The measurement of ammonia formed by glutaminase action on glutamine in blood requires an accurate microdetermination of ammonia. Conway's microdiffusion technic (6) was employed with modifications. One milliliter of 0.0050N HCl is placed in the center well. The outer well contains aliquots for NH_3 analysis and 1 ml. of saturated K_2CO_3. The cell is sealed and incubated for 45 minutes at 38°. The HCl from the center well is transferred quantitatively to a 3-ml. vial, using approximately 1 ml. of NH_3-free water to assist in the transfer. This solution is then titrated to *p*H 7.5 with $Ba(OH)_2$, using an electrometric titration assembly as described by Seligson (7).

RESULTS AND DISCUSSION

Each homogenate preparation is analyzed for glutaminase activity and free NH_3. It has been noted that the enzyme preparation progressively shows a decrease in glutaminase activity and an increase in free NH_3.

Table 1 gives glutaminase activity and free NH_3 values over a period of 1 month.

Table 1. GLUTAMINASE ACTIVITY AND FREE-NH_3 RATE

Day	*μM of NH_3/100 mg. of dry tissue per hour*	*Free NH_3 (μG)*
Initial	176	3.0
0	176	3.0
4	168	4.5
11	155	5.0
23	118	15.0
31	97	25.0

If the free-NH_3 value exceeded 15 μg. per 0.5 ml. of homogenate, the use of this mixture was discontinued because high NH_3 values decreased the accuracy of the determination. The glutaminase activity listed here is lower than that reported by Rector, Seldin, and Copenhaver (5). In this procedure incubation was at *p*H 7.5 for 1 hour, instead of *p*H 8.0 for 30 minutes. These differences account for the lower activity; however, this preparation does give enzymatic action suitable for the determination of glutamine in blood. Plasma from normal humans did not show any glutaminase activity using this method.

Table 2 shows the per cent conversion of glutamine to glutamic acid plus NH_3. This range represents the values obtained from at least five daily analyses over the period of one month. Commercial glutamine is known to contain impurities, therefore, correction for NH_3 liberated without the addition of the enzyme preparation was subtracted from the amount produced with the enzyme. The 1- and 2-μM/ml. standards are of the order of glutamine in blood and show a conversion range of 95–105 per cent. Standards must be analyzed with each run to determine variations in the effect of the enzyme as a result of preparation, age, or incubation time.

Table 2. PER CENT CONVERSION OF GLUTAMINE STANDARDS

Glutamine Standards	*Glutamine* mg./100 ml.	*% Conversion* (Range)
1 μM/ml.	14.6	95–105
2 μM/ml.	29.2	95–98
3 μM/ml.	43.8	75–90
4 μM/ml.	57.4	60–80
5 μM/ml.	72.0	50–70

The level of glutamine in 100 ml. of fasting dog or human plasma is 5–12 mg., as reported by Hamilton and Archibald (1) using different

procedures. Hamilton used the ninhydrin–carbon dioxide method, and Archibald used a glutaminase method. The method described herein gave results ranging from 8.0 to 11.1 mg. per 100 ml. of plasma for normal humans. Hamilton concluded also that red cells have approximately the same concentration of glutamine as the surrounding plasma. This was confirmed using this method. Table 3 shows comparison of the two sources.

Table 3. GLUTAMINE IN MG./100 ML.

Whole blood	*Plasma*
11.6	10.8
11.4	10.5
11.5	10.4

Figure 1 shows a comparison of results obtained in the plasma of normal persons, patients with liver disease, and patients with liver coma after glutamic acid infusion. Patients with liver disease showed high values

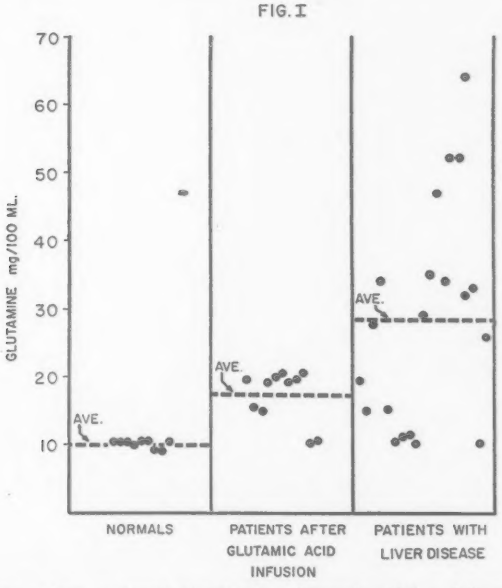

Fig. 1. Mean values ± standard deviation: Normals—10.4 ± 0.64 mg./100 ml.; Patients with severe liver disease—28.2 ± 9.8 mg./100 ml.; Patients after glutamic acid infusion—16.7 ± 2.0 mg./100 ml.

ranging from 10.8 to 60.0 mg. of glutamine per 100 ml. of plasma. These samples were analyzed after 3 months' storage in a deep freeze. Seegmiller and associates (4) obtained a mean value of 8.55 mg. of glutamine per 100 ml. of plasma for normal humans, and 10.3 mg. per 100 ml. for patients in hepatic coma. Although they concluded that patients in hepatic coma are within the normal range, 45 per cent of their patients had glutamine values outside two standard deviations of their normal range. In the present study 75 per cent of the values in liver disease were outside two standard deviations of the normal range. This discrepancy is due to at least two factors. The variation of the normal values is less in the present study (standard deviation 0.64 mg./100 ml.) than in the study of Seegmiller *et al.* (0.98 mg./100 ml.). In addition, four of the values (20 per cent) in the present study are far above the range observed for the other values or the range of Seegmiller. These determinations are on 2 patients who died of their disease within 24 hours of the study and may represent more severe disease than the others or the patients of Seegmiller.

Glutamic acid (8) and ammonia (9) are elevated in the blood in patients with liver disease and glutamine is elevated in the tissues (10). Both glutamic acid and ammonia are removed by the formation of glutamine (11). Therefore it was not unreasonable to search for an elevated plasma glutamine level in liver disease. That the glutamine was not significantly elevated after the infusion of glutamic acid was, therefore, an unexpected finding.

Asparagine was added to standard glutamine solution to determine if rat kidney glutaminase would liberate NH_3 from asparagine. Results indicate that asparagine in the range of 1–10 μM does not yield NH_3 when treated with rat-kidney glutaminase. Archibald (1) has shown that ammonia is formed from adenosine and guanosine by dog-kidney glutaminase. Such experiments were not performed with the rat preparation, but similar behavior toward the purines is to be expected. However, these substances are not present in significant quantities in normal blood.

Ultrafiltrates of blood were obtained and the glutamine measured to determine if the liberated NH_3 obtained from plasma was derived from glutamine or nonfilterable precursors. Analysis of the filtrate gave values of 12.1 to 13.0 mg. of glutamine per 100 ml. of filtrate.

Recovery of glutamine from blood gave the values listed in Table 4. The "glutamine-found" column represents the difference between total glutamine and the plasma level (11.0 mg./100 ml.).

Table 4. Recovery Experiment

Run	Glutamine Added mg./100 ml.	Glutamine Found mg./100 ml.	% Recovery
1	13.2	14.0	106
2	27.3	26.7	98
3	34.8	35.6	102
4	26.2	24.6	94

An attempt was made to determine glutamine in urine by this procedure, but high NH_3 values obtained from urine made it impractical. The procedure for measuring glutamine and glutaminase in blood as presented here is believed to be a simple and accurate procedure. The ready availability of rat-kidney homogenate makes this a convenient method.

SUMMARY

A convenient and accurate procedure for the determination of glutaminase activity and glutamine in blood has been described.

Kidney homogenate from rats made acidotic by feeding NH_4Cl was used as a glutaminase source and the Conway microdiffusion method was used to determine ammonia.

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Nitrofurantoin Estimation in Urine with the Aid of Chromatography

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THE DETERMINATION OF NITROFURANTOIN concentration in the urine of patients receiving Furadantin® [N-(5-nitro-2-furfurylidene)-1-aminohydantoin] for the therapy of urinary tract infections may be desirable for establishing the suitable dosage schedules. The present paper describes a chromatographic method, adapted from previous work at this laboratory (1), for separating interfering substances from Furadantin so that this compound may be determined colorimetrically or spectrophotometrically. Without some procedure for the separation and purification of the nitrofurans, spectrophotometric methods (2) applied to urine are subject to large errors because of the unavailability of true control urines. This is particularly true in the case of abnormal clinical urines where blood, excessive bile pigments, or medication may be present. The colorimetric method for Furadantin (3) cannot be used on untreated urine samples due to the presence of interfering materials. Furadantin concentrations as low as 1 or 2 mg. per 100 ml. can be determined by the chromatographic method to be described.

MATERIALS AND METHOD

Materials

1. Glassware—chromatographic columns 9 mm. (internal diameter) by 150 mm. with the bottom section connected by ground joint were used. A simple substitute of Pyrex glass tubing 9 mm. by 190 mm. may be made with three indentations near the bottom to support cotton plugs and column material. A 250-ml. filtering flask with stopper supports the

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column. The eluates may be collected into vials or short tubes set inside the filtering flasks.

2. Filtrol—Grade 19 from Filtrol Corporation, Los Angeles, Calif.

3. Celite—No. 545 from Johns-Manville Products Corp., Celite Division, New York, N. Y.

4. Dilute Ammonium Hydroxide—10 ml. conc. aqueous NH_4OH (28.1% NH_3) to 100 ml. with water.

5. 0.1M Trisodium phosphate.

6. 1N Hydrochloric acid.

7. 5N Hydrochloric acid.

8. Toluene—reagent grade.

9. Phenylhydrazine hydrochloride (reagent grade)—1.5 Gm. per 100 ml. of water. Prepared fresh daily and refrigerated when not in use.

10. Crystalline standard Furadantin (Eaton Laboratories).

Procedure

The chromatographic column is packed with a small plug of absorbent cotton followed by a layer of Celite, about 5 to 10 mm. deep. On this is packed the following Filtrol-Celite mixture to a depth of about 50 to 75 mm.: One part of Filtrol (0.5 Gm.) is added to two parts Celite (1.0 Gm.) and this mixture is made into a slurry in a beaker with water and is allowed to stand about 5 minutes for swelling. The slurry is then poured into the column while mixing and without interruption under gentle suction. The column is washed with at least 50 ml. of distilled water, with the use of reduced pressure (about 300 to 450 mm. Hg). Under these conditions a column should flow at a rate of about 4 to 6 ml. per minute. A reservoir such as a funnel connected by rubber tubing to the top of the column facilitates the operations. If a column runs too slowly (2 ml. per minute or less) it should be discarded and another one made.

The urine to be chromatographed is diluted 1:5 with water. If the diluted urine contains sediment or is cloudy, it should be centrifuged or filtered through a loose plug of cotton before it is added to the column. If a column begins to run slowly after urine has been added, stirring the top surface with a stiff wire will sometimes restore a higher flow rate. A measured volume (20 ml.) of the diluted urine sample is passed through the column. Following the urine, the column is washed successively with 20 ml. of distilled water, 5 ml. of dilute NH_4OH, and 20 ml. of water, and then the Furadantin is eluted with 0.1M Na_3PO_4 solution. The bright yellow color of the nitrofuran produced by the NH_4OH fades during the subsequent water wash. A brown urinary pigment which is not Furadantin usually is eluted by the NH_4OH wash. About 4 ml. of the first colorless

eluate is discarded and then collection is made in a small vial containing 1 ml. N HCl placed in the suction flask. The collection is continued until the eluate is no longer yellow and at least 1 ml. of uncolored solution follows. The volume of this eluate may vary from 8 to 18 ml., depending on the amount of Furadantin adsorbed on the column. During adsorption and in the subsequent steps, all Furadantin solutions should be protected from strong light, particularly from fluorescent lamps or sunlight. Eluates are usually crystal clear. If a cloudy eluate results, it is centrifuged until clear. The solution is then adjusted with HCl to *p*H 6.8, diluted to 20 ml., and the amount of Furadantin determined either spectrophotometrically or colorimetrically.

Spectrophotometric Determination

The absorbance of the adjusted eluate is determined at 375 mμ (the absorption maximum for Furadantin at *p*H 6.8)[1] with the Beckman Model DU or other suitable spectrophotometer. With a 20-ml. sample of a 1:5 dilution of urine the concentration is calculated by the formula

$$\text{Furadantin, mg. per 100 ml.} = \frac{\text{Absorbance} \times 5 \times 1000}{753}$$

since the $E^{1\%}_{1\text{cm.}}$ for Furadantin at 375 mμ is 753. If dilution of the eluate is necessary, this must be included in the calculation.

Colorimetric Determination

The colorimetric method is based on the formation of 5-nitrofurfural phenylhydrazone, its extraction and estimation (3). Three ml. of the neutralized eluate is transferred to a 18- × 150-mm. lipless test tube, followed by 1 ml. of the phenylhydrazine solution and 1 ml. of 5N HCl. After mixing, the tubes are heated 25 min. at 70° and then placed in cold water for 5 minutes. Toluene (5 ml.) is added, the tube closed with a plastic stopper[2] and shaken vigorously 20–30 times. After centrifugation, the toluene layer is removed and its absorbance at 430 mμ determined. The amount of Furadantin present in the sample analyzed is read from a standard curve of the absorbances of known Furadantin solutions (i.e., 0.2, 0.4, 0.8 mg. per 100 ml.) treated in the same manner. For these readings any colorimeter capable of maximum transmission at 430 mμ and with a band width no greater than 35 mμ is suitable. A Fisher Electro-

[1] The wavelength of the absorption maximum of Furadantin varies with the *p*H of the solution, from 367.5 mμ at low *p*H values to 390 mμ at *p*H 9–10.

[2] Polyethylene stoppers from 3-dram vials closed these tubes tightly. Stoppers with vials were purchases by catalogue No. V5225 from Scientific Glass Apparatus Co., Inc.

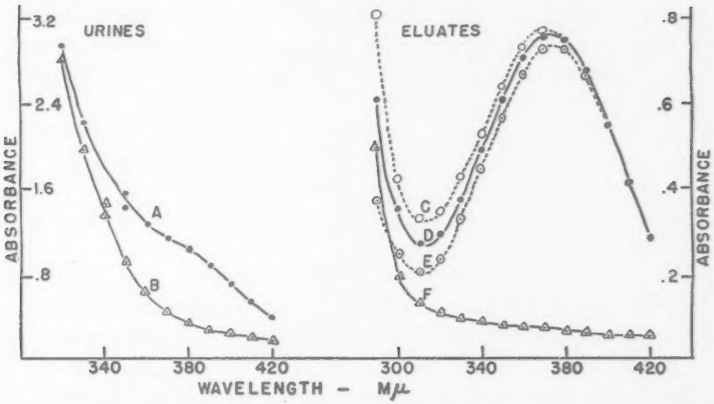

Fig. 1. Ultraviolet absorption curves illustrating the effect of chromatography upon a normal urine containing added Furadantin. Curve *A*, urine with 2 mg. per 100 ml. added drug. Curve *B*, urine with no added drug. Curve *C*, an eluate (diluted to 20 ml.) from this urine with 1 mg. per 100 ml. added drug, after chromatography. Curve D, an eluate (diluted to 40 ml.) from similar treatment, except urine contained 2 mg. per 100 ml. Curve *E*, an eluate (diluted to 400 ml.) from similar treatment, except urine contained 20 mg. per 100 ml. Curve F, an eluate (diluted to 20 ml.) from a similar treatment, except urine contained no drug.

photometer, a Bausch and Lomb "Spectronic-20" Colorimeter, and a Beckman Model B or Model DU Spectrophotometer have been used successfully.

The Furadantin concentration in the sample is calculated as follows:

$$\text{Furadantin, mg. per 100 ml.} = \text{conc. from curve (mg. per 100 ml.)} \times 5$$

If dilution of the eluate is necessary this must be included in the calculation.

RESULTS

Direct quantitative spectrophotometric analysis of Furadantin in urines is not possible because of interference from other substances. This is illustrated by the spectrophotometric curve for a urine containing added Furadantin (2 mg. per 100 ml.) compared with the curve for the control urine (see curves A and B, respectively, Fig. 1).[3] At this level, the presence of the drug cannot be identified by the ultraviolet absorp-

[3] The Furadantin was added as a solution in dimethylformamide (DMF) to avoid undue dilution of the urine. This solvent in the amount used (0.5 ml. with 24.5 ml. urine) had no effect on chromatography or on the subsequent analysis of Furadantin.

tion characteristics of the diluted urine. Following chromatographic treatment the eluate of the urine with added Furadantin (curve D, Fig. 1) allows ready identification as well as quantitative determination of the drug. Eluates (suitably diluted) from other levels (1 and 20 mg. per 100 ml.) of the drug added to urine and subjected to chromatographic treatment are illustrated by curves C and E. These absorption curves (C, D, and E) coincide closely with that of the pure compound in water at *p*H 6.8. The eluate of the control urine (curve F) contains little interfering material.

Good recoveries of Furadantin added to urine at concentrations of 0.5 to 100 mg. per 100 ml. were found by the colorimetric determination following the chromatographic procedure (Table 1). In the case of three urine samples (1, 2, and 4), 20 ml. of urine was passed through a column undiluted and the eluates were made up to 20 ml. In the case of the remaining samples (3, 5, 6, and 7) with higher concentrations, the urines were first diluted 1:5 and then 20 ml. was chromatographed. These eluates were also made to 20 ml. and, in addition to this dilution, another dilution of 1:10 was made when needed to bring the concentration of the solution for analysis in the range of the standards. The colorimetric method may not be applied directly to urine as indicated by the data of Table 1 (samples 8 and 9), unless a high concentration of Furadantin is present, so that interfering substances are reduced by dilution (sample 10).

Table 1. Recovery of Furadantin Added to Urine and Determined Colorimetrically before and after Chromatography

Sample No.	*Sample description*	*Furadantin added (mg./100 ml.)*	*Furadantin found (mg./100 ml.)*
1	Control urine, chrom.[a]	None	0.01 to 0.04[c]
2	Urine + drug, chrom.	0.5	0.49
3	Urine + drug, dil. 1:5, chrom.	1.0	1.1
4	Urine + drug, chrom.	2.0	1.8
5	Urine + drug, dil. 1:5, chrom.[b]	20.0	20.7
6	Urine + drug, dil. 1:5, chrom.[b]	50.0	51.1
7	Urine + drug, dil. 1:5, chrom.[b]	100.0	98.2
8	Control urine, not chrom.	None	2.01[c]
9	Control urine, not chrom., dil. 1:6	1.0	4.16
10	Control urine, not chrom., dil. 1:100	100.0	108.0

[a] A 20-ml. sample of urine or its 1:5 dilution was passed through a column for each chromatographic experiment.

[b] This eluate was also diluted 1 to 10 before colorimetric determination.

[c] The total absorbance for each control urine has been estimated as Furadantin.

DISCUSSION

The solubility of Furadantin is greater in urine than in water and varies with the *p*H of the solution. In water at *p*H 4.8 the solubility is 12 mg. per 100 ml., whereas at *p*H 7.0 it is 19 mg. per 100 ml. Concentrations of 66 and of 90 mg. per 100 ml. have been attained by saturating urine with Furadantin at *p*H 7.0 and 7.3, respectively.

The concentration of Furadantin in a urine sample should be less than 20 mg. per 100 ml. before passing the sample through the chromatographic column. The preliminary 1:5 dilution has been included in the method to reduce all Furadantin concentrations to this range. Richards and coworkers (4) found urinary Furadantin concentrations of 12.5 to 50 mg. per 100 ml. in subjects receiving 100- to 200-mg. doses of the drug. In our experience (5), maximum urinary concentrations ranging from 4 to 48 mg. per 100 ml. were observed following single 100-mg. doses to normal individuals. Beutner and associates (6) reported that persons receiving 150 mg. 4 times a day exhibited maximum urinary concentrations of 20 to 46 mg. per 100 ml. For certain investigations it may be desirable to determine lower concentrations (1 mg. per 100 ml.) of the drug in urine. In such an event, the preliminary 1:5 dilution of the urine may be omitted.

In using the spectrophotometric method on chromatographed eluates, no interference was found in the presence of chloramphenicol, penicillin G, sulfisoxazole, bilirubin, blood, or blood pigments. However, interference was observed in the presence of tetracycline, chlortetracycline, or oxytetracycline. These substances apparently accompany Furadantin on the chromatographic column and exhibit ultraviolet absorption in the same range (375 mμ).

The colorimetric method has been found satisfactory in the presence of chlortetracycline, chloramphenicol, and oxytetracycline (7). Tetracycline also does not interfere in this analytic procedure. The colorimetric method does not require a spectrophotometer for light-absorption measurements and is less subject to interference. The spectrophotometric method is somewhat quicker if a suitable instrument is available and if tetracyclines are known to be absent. Either method is satisfactory in the presence of high concentrations of Furadantin.

SUMMARY

A method for the determination of Furadantin in urine has been described. Furadantin is separated from interfering urinary pigments by a

chromatographic adsorption technic. Furadantin concentration may then be determined spectrophotometrically from the absorbance (375 mμ) of the eluate, or the eluate may be further treated to form the phenylhydrazone of nitrofuraldehyde from Furadantin and the determination carried out colorimetrically.

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Clinical Chemistry Work-Load Control Factors

Edmund P. Finch, Seward E. Owen, and Walter E. Byers

WORK-LOAD CONTROL FACTORS in clinical laboratories are not available in the literature. Business engineering and manpower-study methods are not readily applicable to laboratory work in which varied human elements exist. The remarkable variety of methods, choice of technics, personal viewpoints, lack of contacts, and few chances for direct comparison with the work of others in the field also make meaningful comparative work-load studies difficult to produce. It is believed the results of our studies will be of interest to others and stimulate comparative studies.

METHODS

The basis of our methods are the preparation of daily load sheets of work done in each specialty. These load sheets are then used as a basis for the construction of tables, graphs, and data. These study methods have been used in our laboratories for a period of years. They have served as guides in staff adjustments and in the setting of work-load limits, as an indirect index of accuracy and final completeness of the work in the various specialties, and as graphs for reference use in manpower studies, budget costs, etc.

Certain basic procedures must first be adopted before attempting analysis. An example of the guides we have used: Only those workers most directly concerned with, and performing, diagnostic tests are involved. For example, the staff of one section in clinical chemistry includes 8 to 10 workers; these are the section chief, the assistant section chief, 5 to 7 technicians, and a glassblower. This section serves a large

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general medical and surgical hospital and provides service for approximately 25,000 patient-days per month. There is also an extensive resident-training and consultant program and many research projects are in process. A second complete clinical chemistry section with an average of 4½ workers serves patients on the chronic-disease wards. This laboratory services the neurology, psychiatric, and tuberculosis services, and provides coverage for approximately another 25,000 patient-days per month. All staff workers are on a 40-hour week. Three regular shifts are used and week-end coverage is rotated at the general medical and surgical hospital. All wards are provided laboratory service for 168 hours each week. Laboratory staff members do not usually work nights or Sundays at the second section. All paid time (including vacations or leave) is included in our studies. In the determinations of over-all workload figures for the entire laboratory system, all staff members except pathologists, administrative staffers, secretaries, janitors, and morgue attendants are included. Statistical studies involving costs or budget affairs include all assigned staff members. Some budget or operating reports have also included depreciation, maintenance and utilities, estimated interest on property valuations, insurances, taxes, and other essentials as salaries and supplies.

As a guide to what constitutes a single reportable test, the specifications are strict and well defined in a lengthy technical bulletin for laboratories in hospitals of the Veterans Administration. Examples are: (1) A routine urinalysis is one test although it includes color, reaction, specific gravity, albumin, sugar, and microscopic studies. (2) Diagnostic tests requiring a series of determinations such as the glucose tolerance, urea clearance, and others count as one test only. (3) Repeat tests for the purpose of checking or verification are not counted at all. (4) Not included in the test count are the routine laboratory preparations, standardizing equipment or reagents, cleaning glassware, checking in supplies, attending seminars or conferences, venipunctures or specimen collections, preparation of laboratory reports, and other time-consuming duties.

Control studies on a standard laboratory hour for each section are also used in analyzing time for the technical and nontechnical duties and in work-simplification adjustments. The standard laboratory hour is used as a unit in this report. For clinical chemistry the standard laboratory hour contains time factors for the following: technical work at the bench on specimens—44 minutes; specimen-collection time—5.3 minutes; rest and personal needs (includes vacations)—6.7 minutes; other nontechnical time (includes report preparation, telephone reports, attendance

at conferences and seminars, reagent and equipment preparation, etc.) —4.0 minutes. The breakdown of the standard laboratory hour will, of course, vary with the specialty and with the laboratories since many factors influence this figure.

RESULTS

When urinalysis procedures are combined with those of clinical chemistry, an unrealistic figure is obtained. This is shown in Table 1. This table presents the total Clinical Chemistry and total urinalysis diagnostic tests (C-U.T/M/HR—Chemistry-Urinalysis Tests per man per hour); the chemistry tests along (C.T/M/HR—Chemistry tests per man per hour); and the urinalysis test alone (U.T/M/HR. Urine tests per man per hour). At the laboratory serving the general medical and surgical unit, 6.3 chemistry plus urine tests were done each complete standard laboratory hour. Chemistry tests alone averaged 4.7 tests per standard laboratory hour, while urine tests alone averaged 13.3 per standard hour. At the laboratory serving the chronic-disease sections, chemistry and urine tests averaged 6.5 tests per standard man hour, chemistry alone was 3.1 and urinalysis averaged 19.9. The unit serviced by this laboratory is located in a physical plant similar to an Army General Hospital. Other factors influencing work-load output are given in the discussion. The average number of tests per standard laboratory hour, over a 3-year period for all specialties of the two laboratories, was 5.7. This figure varies considerably with each specialty.

A distribution of clinical chemistry tests for 1 month is shown in Fig. 1. This division is typical and is of interest because the ratio of abnormal to normal test results is shown for various chemical tests.

Table 1. Test Load Factors for the Chemistry and Urinalyses Sections

Time	*Chemistry*	*Urinalyses*	*C-U.T/M/HR*	*C.T/M/HR*	*U.T/M/HR*
		Laboratory 1			
1953	98,514	62,827	6.5	4.7	15.1
1954	86,589	57,485	6.6	5.0	13.6
1955	75,421	46,307	5.9	4.5	11.3
Average	86,841	55,524	6.3	4.7	13.3
		Laboratory 2			
1953	27,094	41,831	6.6	3.3	20.1
1954	25,025	43,040	6.5	3.1	20.7
1955	20,108	38,836	6.3	2.8	18.7
Average	24,076	41,236	6.5	3.1	19.9

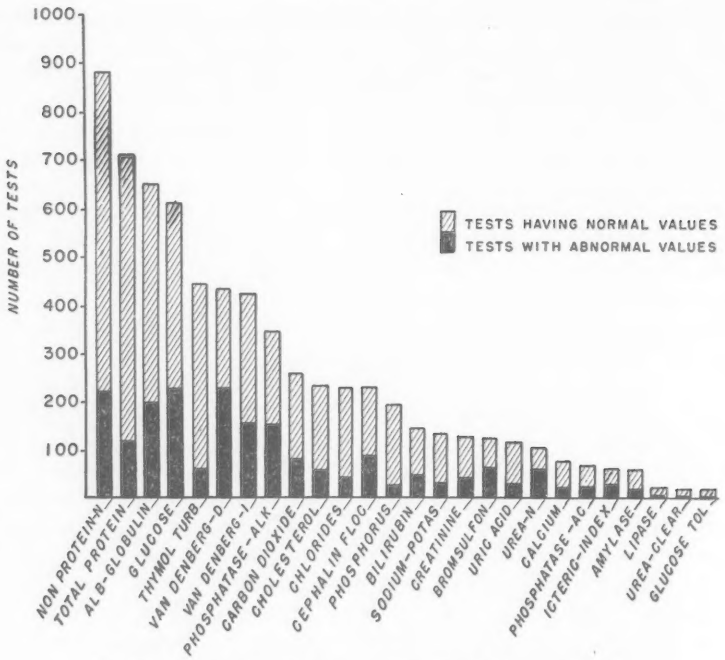

Fig. 1. Clinical chemistry tests. Distribution for one month.

DISCUSSION

Many factors have been found to affect the work-load output in clinical chemistry. In general, those factors which produce major differences would remain fairly constant for each individual laboratory. Some of the factors influencing work-load output include laboratory location, laboratory layout and equipment, physical plant of the hospital, traffic plans, types of hospital service offered, amount of work in each specialty, general types of diagnostic procedures, manner of specimen preparation (whether technicians or others collect specimens), meal hours, clarity and timing of laboratory work requests, amount of interruptive emergency work, and many others, not the least of which is the ability of the supervisor to coordinate and integrate the laboratory work program. Affecting work output also are many other items. Some of these are most difficult to describe as they include motivation, the development of attitudes, effective communication, training, public relations, and a knowledge of basic human needs.

SUMMARY

An analytic study of work-load factors in clinical chemistry shows that there are many elements involved. An average of 6.3 to 6.5 tests per man hour were obtained when clinical chemistry and urinalyses were combined. For clinical chemistry alone the tests per man hour averaged 3.1 in a hospital servicing patients with chronic diseases and 4.7 in a general hospital. The breakdown of a standard laboratory man hour in clinical chemistry included 49.3 minutes for the collection and determinative tests; the remaining time included vacations, training, personnel needs, nontechnical duties, and other items.

Semimicro Method for the Determination of Salicylate Levels in Blood

J. I. Routh, W. D. Paul, Emilio Arredondo, and R. L. Dryer

A WIDE VARIETY OF METHODS are available for the determination of salicylate in body fluids and tissues. These range from the spot test type used at the patients' bedside to the organic solvent partition methods employed in more exact studies. In recent years, the method of Brodie *et al.* (1) involving extraction with ethylene chloride has gained widespread acceptance. The method commonly employs 1 or 2 ml. of plasma and can be applied over a wide range of salicylate concentrations. P. K. Smith *et al.* (2) adapted the method to the determination of urinary salicyluric acid and salicylates.

In the course of our investigations we have had occasion to determine salicylate levels in all types of body fluids, whole blood, plasma, serum, and tissue samples. At times we have been handicapped by low salicylate levels, insufficient available volume of samples, and necessity for frequent sampling. The renewed interest in the course of blood salicylate levels at various time intervals following a dose of aspirin also posed a similar problem.

The majority of investigators have determined salicylate levels 30 minutes to 1 hour after the ingestion of aspirin. We have long felt that it would be of interest to study levels that occur within the first few minutes after the ingestion of the drug. Such an experiment would involve large numbers of patients to obtain venepunctures at staggered intervals. If more frequent specimens could be taken from the same patient, additional information could be obtained concerning salicylate levels versus dosage versus time.

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EXPERIMENTAL

Early in the study it was decided to use a fingertip blood specimen of 0.2 ml., an amount often employed in micro blood-sugar determinations. Laboratory personnel are experienced in obtaining fingertip blood with a minimum of discomfort to the patient, and it is a relatively simple matter to obtain multiple specimens.

Since the 6N hydrochloric acid used in the Brodie method serves both as a precipitant of blood proteins and as an acid agent to force the salicylate into the ethylene chloride, our first efforts involved the selection of an acid precipitant. Various concentrations of hydrochloric, tungstic, sulfuric, phosphoric, phosphotungstic, molybdic, phosphomolybdic, molybdotungstic, and perchloric acids were studied. Standard curves were prepared employing aqueous solutions of salicylates plus these agents. Recovery of added salicylate from plasma and whole blood was also investigated. It was found from this series of experiments that low blanks and the best recoveries could be obtained when either hydrochloric acid or sulfuric acid served as the precipitating agent. Since recovery of added salicylate was more quantitative using hydrochloric acid the method and the results from the use of this agent will be described.

METHOD

Step 1. An 0.2 ml. specimen of blood, serum, or plasma was expelled under the surface of the precipitating solution (0.3 ml. 6N HCl and 0.5 ml. H_2O) in a 12-ml. glass-stoppered centrifuge tube. The pipet was rinsed several times with the solution; 6 ml. of ethylene chloride was added, and the tubes were shaken for 5 minutes in a mechanical shaker.

Step 2. The tubes were centrifuged and 5 ml. of the ethylene chloride layer transferred to similar tubes containing 3 ml. water and 0.1 ml. ferric nitrate solution (1% $Fe(NO_3)_3$ in 0.07N HNO_3).

Step 3. The tubes were again shaken for 5 minutes, centrifuged, and the water layer was read in a Beckman DU spectrophotometer at 540 mμ.

Screw-top culture tubes, 17 mm. x 125 mm. with Teflon-lined caps, and 30-ml. glass-stoppered bottles were used instead of the glass-stoppered centrifuge tubes in earlier studies. The culture tubes had a tendency to leak on prolonged use, whereas it was difficult to remove a sufficient quantity of the water layer from the bottle to fill a standard Beckman cuvet. A wooden test-tube rack holding 24 tubes fitted with a snap-lock, hinged cover lined with sponge rubber was used for convenient shaking of the centrifuge tubes.

The fingertip blood specimens were collected in 0.2-ml. pipets. After

centrifugation in step 2 the protein precipitate collected as a layer between the aqueous and solvent phase. To obtain 5 ml. of the lower ethylene chloride layer without contamination by precipitated protein it was necessary to tip the disc of precipitate to one side or to break a hole through the protein layer. This manipulation was carried out conveniently by the use of slender rod-shaped wooden applicators. In step 3 little difficulty was experienced from the formation of emulsions in the aqueous layer. Hydrochloric acid did not produce emulsions, although occasionally they resulted if sulfuric acid was used. Such emulsions were easily broken by tapping the tubes and recentrifuging.

The standard 10-mm. light-path Beckman cuvets requiring approximately 2.5 ml. of solution for reading were used in the early stages of the investigation. A shift to micro cuvets in which approximately 1.0 ml. of solution can be read minimized the problem of obtaining a sufficient volume of the aqueous solution in step 3. Although 3 ml. of water was commonly used in step 2 it is possible to decrease this to 2 ml., thereby increasing the absorbance of the final colored solution.

Standard curves were prepared and recovery experiments carried out with each of the precipitants. Color intensity developed from standard solutions and from blood or plasma plus added salicylate was similar for the two agents but was maximal with hydrochloric acid. For this reason typical results to be reported are based on the use of hydrochloric

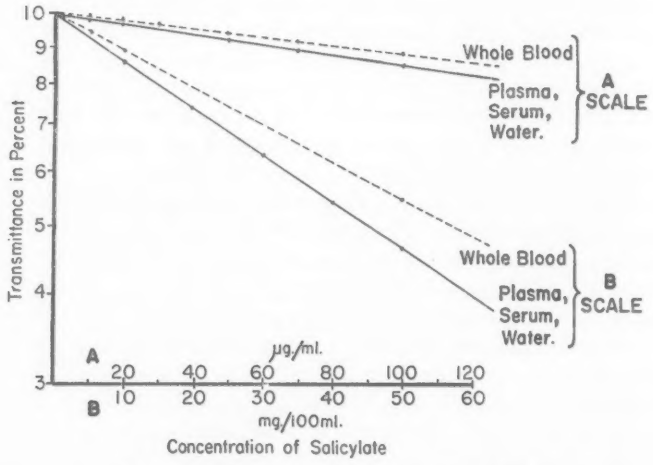

Fig. 1. Calibration curves for the macro method.

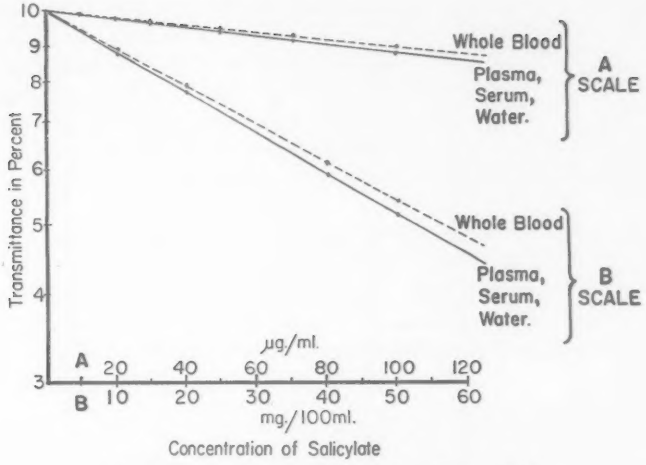

Fig. 2. Calibration curves for the micro method.

Table 1. PLASMA AND WHOLE BLOOD SALICYLATE BLANKS

Micro method		*Macro method*	
Plasma	*Blood*	*Plasma*	*Blood*
0	1	2	0
2	0	0	0
0	0	0	1
0	1	2	0
1	6	0	0
0	0	4	0
0	1	0	7
5	7	5	7
4	1	1	9
2	0	0	3
5	0	2	5
6	0	2	1
1	7	0	8
7	0	0	0
4	0	6	0
0	5	0	1
2	3	0	0
0	7	1	0
2	1	0	0
0	1	6	7
$\bar{X}$ = 2.0	$\bar{X}$ = 2.1	$\bar{X}$ = 1.6	$\bar{X}$ = 2.5
S = 2.3	S = 2.7	S = 2.0	S = 3.2

All values are expressed in µg./ml., $\bar{X}$ is the sample mean, S is the sample standard deviation.

Table 2. SALICYLATE LEVELS ON FINGERTIP AND VENOUS BLOOD AND PLASMA SPECIMENS

Patient	*Micro method*			*Macro method*	
	Fingertip	*Venous*	*Plasma*	*Venous*	*Plasma*
W	6	6	13	16	15
W	22	15	27	20	25
S	9	15	9	20	14
H	6	8	1	7	6
G	4	1	0	8	2
B	1	1	0	6	0
H	26	31	35	32	23
L	5	5	1	2	6
H	54	51	43	42	56
S	22	8	16	23	12
T	21	22	..	28	28
Sc	10	13	..	19	12
L	13	12	..	14	5
D	19	24	..	28	26
Se	23	13	..	19	13
P	27	20	..	27	25
H	24	20	..	24	20
Sm	31	35	..	36	23
A	30	40	..	34	31
S	30	40	..	30	30
P	27	40	..	40	32

All values are expressed as μg/ml.

acid. Such a procedure will also facilitate comparison of results of the micro method with the standard macro procedure of Brodie *et al.*

A series of standard curves prepared by the macro method comparing water solutions of salicylates, blood plus salicylate, and plasma plus salicylate are shown in Fig. 1. As stated earlier we are interested in documenting events that occur shortly after the ingestion of aspirin and therefore require a method capable of measuring low levels of salicylate 0 to 100 μg./ml., as well as higher levels, from 0 to 50 mg./100 ml.

Similar experiments were conducted using the micro method producing curves shown in Fig. 2.

To obtain accurate values of salicylate concentrations at the low levels frequently encountered in our studies it was important that the method produce zero or extremely low absorbance readings for blood and plasma blanks. Salicylate blanks were run on specimens from hospital patients, laboratory workers, and medical students by the macro and micro technics. Typical results of these studies are shown in Table 1. The data in this table are random samples of a larger series.

Since the determination of the standard curves for plasma, serum, and

whole blood involved the addition of known amounts of salicylate to these substances, a comparison of these curves with those of water solutions of salicylate provides a measure of the recovery of added salicylate. An examination of Figs. 1 and 2 indicates substantially complete recovery of salicylate added to plasma or serum in both the micro and macro method. Recovery from whole blood is more nearly complete in the micro method and is less satisfactory at higher salicylate levels in the macro method.

In our studies we have had occasion to extensively compare the micro and macro technic using fingertip blood, venous blood, plasma, and serum specimens. Table 2 presents typical results obtained on a variety of specimens by the micro and macro methods 10 to 20 minutes after a 10-grain dose of aspirin. The data in this table are random samples of a larger series.

DISCUSSION

Colorimetric methods for the estimation of salicylate levels in body fluids usually involve measurement of the purple color produced by ferric ions in the presence of salicylates. Brodie and his coworkers found that extraction of the specimen with ethylene chloride separated the salicylate from interfering substances and thus developed an accurate method characterized by low blank values (1). In an attempt to by-pass the extraction procedure and shorten the time required for the determination, other investigators have employed so-called single solution methods. Their reagent may consist of protein precipitants and ferric salts, Trinder (3), or a nitric acid solution of ferric nitrate, Keller (4), and the recent modifications of Keller's method reported by Cosmides *et al.* (5). The majority of these latter methods may be capable of determining salicylate concentrations in a range of 10–50 mg./100 ml. but they are inadequate for the estimation of levels obtained within a few minutes after the administration of moderate doses of salicylates (0 to 50 μg./ml.). Since they do not remove interfering substances, blank values for blood or plasma specimens are erratic and high. For example, in a series of salicylate blank determinations employing the Trinder method, "apparent" serum levels varied from 0.7–5.7 mg./100 ml. (7–57 μg./ml.) with an average of 2.6 mg./100 ml. (26 μg./ml.), and "apparent" whole blood levels varied from 1.7–3.5 mg./100 ml. (17–35 μg./ml.) with an average of 2.4 mg./100 ml. (24 μg./ml.). Reported blank values for plasma in the Keller type method average 3.5 mg./100 ml. (35 μg./ml.) with a range of 2.0–5.0 mg./100 ml. (20–50 μg./ml.). It is apparent that the "single solution"

methods are not yet capable of accurately estimating low levels of salicylate.

It should be emphasized that salicylate levels of 0 to 50 μg./ml. in biological fluids are considerably more difficult to determine with the same degree of accuracy than levels of 10 to 50 mg./100 ml. It must also be recognized that in the range of 0–10 μg./ml. corresponding to a transmission range of 95–100 per cent photometric errors become increasingly significant. However, the data of Figs. 1 and 2 showing a greater divergence between the whole blood and plasma curves on the A scale strongly support the contention that there is a real difference in the behavior of the original macro method and the proposed micro method. Comparison of the curves on the B scale, Figs. 1 and 2, illustrates the difference in the two methods even more clearly.

Salicylate blanks in a desirable method should be low and reasonably uniform and the spectrophotometric technic employed must be of high order. The cuvets must be chosen with care and frequently checked for blank values when filled with water or reagents. The use of micro cuvets and photomultiplier tubes increases the sensitivity of the method but at the same time imposes a requirement for greater care in conducting the analyses.

SUMMARY

A micro method for salicylates capable of determining levels of 0 to 50 μg./ml. on fingertip blood specimens has been developed. The method is characterized by low plasma or whole blood blanks, the ability to measure very low salicylate levels, and sufficient range to compare favorably with the macro method of Brodie *et al.* at higher levels.

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Determination of Oxalate in Urine

Estelle R. Hausman, John S. McAnally, and George T. Lewis

THE DETERMINATION of oxalate in urine by permanganate titration has always been troublesome because of the precipitation of other oxidizable compounds along with calcium oxalate. A search for alternative procedures is therefore in order. There has recently appeared a method for the determination of oxalate through its reaction with indole to produce a colored compound which conforms to Beer's law when the intensity is measured in a spectrophotometer (1). In a solution made strongly acid with H_2SO_4, a red or pink color is produced, while if the concentration of H_2SO_4 is reduced, a yellow color is formed. Bergerman and Elliott found that of a number of compounds tested only formic acid reacted similarly.

It was found earlier (2) that formic acid would react with 2-methylindole to give a compound characterized as 2-methylindyl-2-methylindylindenmethane. The free base is yellow in color, while the diformate salt is red. In addition, it was shown that indole and either oxalic or formic acid would react when heated with sulfuric acid to give a red dye (3). It appears that oxalic acid is decarboxylated to give formic acid and the formic acid then reacts with one molecule of indole to form formylindole. This molecule reacts with a second molecule of indole to form indylindolidinemethane. It is upon this series of reactions that the determination depends.

The problem of quantitative measurement of oxalate in urine was found to involve primarily the precipitation of the oxalate ion and the separation from the precipitate of contaminating materials which interfere with the colorimetric measurement.

REAGENTS

Indole reagent: 0.40 Gm. indole (EK 2773) dissolved in 100 ml. 95% ethanol.

From the Biochemistry Department, School of Medicine, University of Miami, Coral Gables, Fla.

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H_2SO_4: Reagent (ACS) grade.

$CaCl_2$: Saturated aqueous solution of reagent (ACS) grade.

NH_4OH, 0.5% by volume: 5 ml. of reagent (ACS) grade concentrated NH_4OH added to 995 ml. H_2O.

Lloyd's reagent:

Oxalic acid standard: Dissolve 1.4870 Gm. sodium oxalate, reagent (ACS) grade, in distilled water and dilute to 1 liter. This is equivalent to 1.0000 Gm. anhydrous oxalic acid. From this prepare a working standard containing 0.5 mg. oxalic acid per 1.0 ml. in 1N H_2SO_4 by the addition of 50 ml. 2N H_2SO_4 to 50 ml. of the stock standard.

PROCEDURE

Twenty-four–hour urine samples were collected in containers to which 10–12 ml. of glacial acetic acid had been added. The samples are stable at room temperature for at least 48 hours, and should not be refrigerated.

The entire urine sample was diluted to 2 L. and the *p*H was adjusted to 4.9–5.1 with concentrated NH_4OH. The amount of NH_4OH required is so small that there is no appreciable increase in volume. After adjustment of the *p*H, 1 Gm. of Lloyd's reagent was added to a 150-ml. aliquot. This mixture was shaken intermittently for 5 minutes, and was allowed to stand for an additional 5 minutes. The treated urine was filtered through Whatman #1 filter paper. The first 10 ml. of the filtrate were collected separately and discarded.

Two 40-ml. aliquots of the filtrate were pipetted into heavy-walled conical centrifuge tubes, 0.2 ml. of saturated $CaCl_2$ were added, and the tubes were refrigerated at 4° overnight. The tubes were then centrifuged in the cold, 2–5°, for 30 minutes at 2000 rpm in the International PR-1 centrifuge. Approximately 30 ml. of the supernatant were decanted and discarded, the precipitate was resuspended by stirring, and the mixture was transferred to a conical 12-ml. graduated centrifuge tube. (It was found necessary to recalibrate these tubes at the 2.0-ml. mark.) The mixture was recentrifuged and the clear supernatant discarded. The tubes were then inverted and allowed to drain for a few seconds. The 40-ml. tubes were rinsed with 3 ml. of ice water, and the washings added to the 12-ml. tubes. The stirring rod which had been used to stir the mixture was rinsed with these washings. The mixture was centrifuged and the supernatant discarded.

The precipitate was washed twice with 2-ml. portions of 0.5% (by volume) NH_4OH. After the second washing the excess ammoniacal

solution remaining on the sides of the tube was removed by blotting with filter paper while the tubes were held in a horizontal position.

One milliliter of 1N H_2SO_4 was added to the precipitate in the tubes, and the tubes were placed in a bath of boiling water for a few seconds. Stirring effected complete solution in most cases. The tubes were cooled, the stirring rod was removed after having been rinsed with a little less than 1 ml. of 1N H_2SO_4. The tubes were centrifuged to get all of the liquid into the tip of the tube. The volume was made up to 2 ml. with 1N H_2SO_4, and the tubes were stoppered, shaken, and centrifuged. (Some samples gave a precipitate which was not completely soluble in 1N H_2SO_4. This precipitate did not give rise to a loss of oxalate in these samples, but it did give rise to an atypical color if it was allowed to get into the color-developing solution.)

Color Development

Samples were prepared for color development as follows (unknown in duplicate, standard in triplicate):

Unknown—1.5 ml. the 1N H_2SO_4 solution + 0.5 ml. indole reagent solution

Standard— 1.0 ml. working standard + 0.5 ml. N H_2SO_4 + 0.5 ml. indole reagent solution

Blank— 1.5 ml. 1N H_2SO_4 + 0.5 ml indole reagent solution

The above were mixed in Klett-Summerson colorimeter tubes. Two milliliters of concentrated H_2SO_4 were added to each tube. This was done by allowing the H_2SO_4 to run down the side of the tube and to form a layer below the oxalic acid–indole solution. After the H_2SO_4 had been added to all tubes, each was mixed slowly with a footed stirring rod so that heat was not liberated too rapidly. The stirring rods were left in the tubes. All tubes were placed in a water bath at 80–90° for 45 minutes, and after removal were cooled in tap water. The volume was made up to 5 ml. with H_2O, and the solutions were mixed by stirring. Some heat was evolved so that it was necessary to cool the tubes a second time. The tubes were allowed to stand at room temperature for 45 minutes before readings were made with a Klett-Summerson photoelectric colorimeter. A #54 (green) filter was used. The color formed is stable for several hours, but it was still found advisable to read the standards at the same time as the unknowns.

RESULTS

Preparation of Standard Curve

As is shown in Fig. 1, the standard solutions follow Beer's Law throughout the working range of the instrument used, the Klett-Summerson

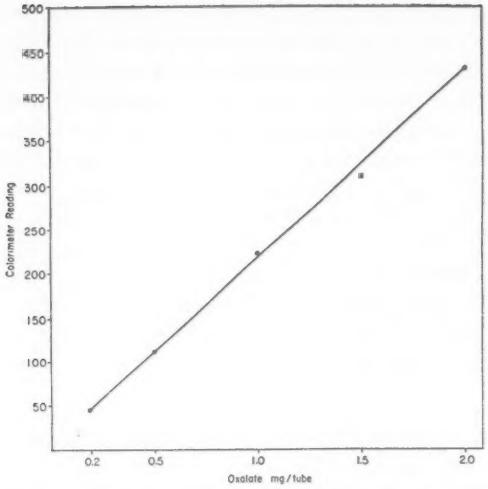

Fig. 1. Standard curve for determination of oxalic acid.

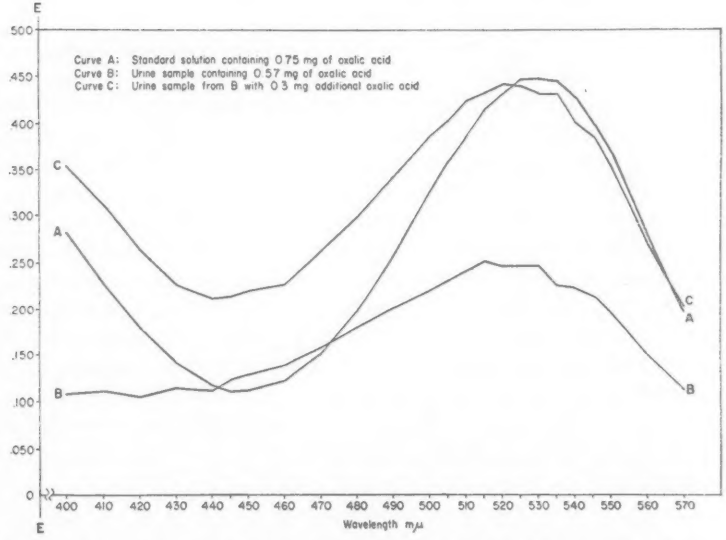

Fig. 2. Absorption spectra of the colored compound produced from oxalic and indole.

photoelectric colorimeter. The selectivity of the method is shown in Fig. 2 were absorption spectra for pure oxalic acid and oxalate precipitated from urine are compared. Since maximum absorption occurs at 525–530 mμ, the filter chosen for colorimetry was that having maximum transmission at 540 mμ.

Oxalic Acid Content of Urine

Several determinations were made on urine samples from three different individuals. The samples were collected as outlined in the experimental procedure. In five instances the oxalate determination was carried out on the same sample on successive days. The results of these determinations are shown in Table 1. Preliminary data on urine samples collected at different periods during the day indicate that there are considerable variations in the rate of oxalate excretion during a 24-hour period.

Recovery of Oxalic Acid Added to Urine

In these experiments, known amounts of oxalic acid were added to aliquots of urine samples and the entire procedure was carried out with aliquots of the original urine and those aliquots to which oxalic acid had been added. As indicated in Table 2, recoveries of added oxalate range from 95–110 per cent.

Table 1. Excretion of Oxalate in Urine per 24 Hours

Subjects		
A	*B*	*C*
21	23	49
33	25	27
19	20	32
30	28	..
12	..	..

Figures are milligrams of oxalate.

Table 2. Recovery of Oxalate Added to Urine Samples

Sample	*Oxalate in urine* (mg.)	*Oxalate added* (mg.)	*Oxalate found* (mg.)	*Recovery* (%)
1	1.85	2.00	3.80	98
2	1.15	1.00	2.20	105
3	2.55	1.00	3.65	110
4	2.35	1.00	3.40	105
5	2.50	1.00	3.45	95
6	1.25	1.00	2.30	105

SUMMARY

A method has been developed for the determination of oxalate in urine. This method has proved superior, in the experience of the authors, to the older titrimetric determination of this urinary component.

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the Clinical Chemist

PRESIDENTIAL ADDRESS

Robert M. Hill

I HOPE I WILL BE PARDONED tonight if, as an officer of the American Association of Clinical Chemists, I direct my remarks primarily toward the members of that organization. There are a few things I would like to suggest to our young Association and I am not sure that I will get another such opportunity. However, as *organized* clinical chemists we are all young—both here and abroad. Perhaps we are all somewhat bedeviled by the hopes and fears of youth, and it may well be that some of what I say, though directed primarily to American clinical chemists, may have wider application.

The events of this week mark a milestone in the short history of the American Association of Clinical Chemists and in the history of Clinical Chemistry in America. The success of the meetings of the International Congress is a splendid tribute to the vision, organizing ability, and tireless devotion to a high purpose of those men who have nursed our young Association from the small beginnings of eight years ago to the vigorous organization that, with their leadership, is now playing host to this great Congress.

We are meeting in the city that is the birthplace of our Association, and I am doubtful that its birth, or at least its birth and continued and healthful growth, could have occurred in many other places. No doubt there are men elsewhere with the vision and as deep a sense of need, but here men with these attributes were associated with a considerable population of like-minded men. Here we have had the organizing genius backed by numbers, cooperation, and group determination.

I wonder if those of you who live and work amidst the clinical-chemical affluence of New York City can appreciate the difficulties under which some of us from far places have had to labor. When I started work in the West in 1925 you could have counted all of the clinical chemists of the Rocky Mountain Empire on the fingers of one hand and you would have had a thumb and a few fingers

Delivered before the Annual Meeting of the AACC held during the International Congress on Clinical Chemistry, September 1956, New York City.

left over. In our very young and struggling medical school hospital at that time the clinical chemistry laboratory had no budget and, with all laboratory work done without cost to the patient, equipment and supplies were what could be purloined from the teaching laboratory. Since that day the advance has been very considerable but in the thinly settled areas of the country the clinical chemist is still a rather lonely person. It is refreshing and reassuring to come to these meetings and find that so many other clinical chemists actually exist.

It is fitting at the national meeting each year that we should recount the accomplishments of our young society. It is a story of which we are justly proud. The simple cataloguing of a few of the more important aspects of our growth should give us enthusiasm and courage for the future.

First, and most important, we *have* organized and have successfully passed through the early days of uncertainty.

Second, we have started a series of publications which we call *Standard Methods of Clinical Chemistry*. In these volumes will be collected laboratory methods for the practice of clinical chemistry, chosen and carefully checked and tested by members of our own organization. The plan to review all methods already appearing in the series, during the preparation of each new volume, is a guarantee that our *Standard Methods* will live and grow with our science. Standard methods we will have, but they will be standard methods that improve as our knowledge advances. These "methods" we confidently expect will soon come to be recognized by all branches of clinical science as *the* standard methods.

Third, we have launched a journal, CLINICAL CHEMISTRY, one that already richly deserves its place among the foremost scientific journals.

New as they are, these things represent great accomplishment. They portend a future bright with promise. Now we may well ask, "How should our future course be plotted in order that we may best realize that promise?" This is a hard question to answer and one that will be answered differently by different, equally able men. And so in what follows, I wish to emphasize that I am offering my own suggestions and I do not know how many will agree with me.

In plotting a course our problems as well as our achievements must be considered. And one problem of which we are all aware is that we are growing up under the shadow of restrictive legislation and restrictive habits of thought and attitude. These things are irritating. But I think we will agree that all types of clinical laboratories must be regulated by restrictive laws in order to keep the irresponsible and the greedy from preying on the public. And since much of this legal edifice is old or has its inception in old ideas or definitions, it is to be expected that a newly emerging specialty such as ours should bump its head on the old, and in some degree outmoded, legal superstructure. I do not think that this should worry us too much, but to cope with it requires patience. Patience, to be sure, is a negative virtue that any man may cultivate, but it is a powerful weapon. And when things are achieved with patience alone then no wrongs have been committed that must later be righted. But usually patience must be forti-

fied with positive attitudes and action, and I envisage three principal courses of action through which our patience may be fortified. And in all three of these each of us can lend a hand.

First, we can acquaint ourselves with the activities of our respective legislatures and exert our influence in channeling legislation away from further limitation of our proper activities as clinical chemists.

The second course of action that I would suggest is concerned with the training of clinical chemists. Our country is in great need of more clinical chemists, and there are only a few schools that offer training toward such a career. Few of the Departments of Biochemistry in our medical schools are headed by men with a major interest in clinical chemistry. In order that a sufficient number of high-caliber young men may be attracted to and trained for careers in clinical chemistry, either new Departments of Clinical Chemistry should be established, or separate Divisions of Clinical Chemistry should be organized in the Departments of Biochemistry already existing. These departments or divisions should be headed by men whose major interest is clinical chemistry, and the graduate instruction and research should be planned toward the Ph.D. degree in that specialty. It may take persuading to stimulate action toward introducing these innovations in our medical schools, but I believe our organization and its members individually would do well to work toward that end.

The third and perhaps the most important course of action by which we may promote the cause of Clinical Chemistry may be simply described as the sincere and unremitting effort to prove by what we *do* that we are indispensable to modern clinical practice. To accomplish this it is our obvious duty to exert ourselves continually toward making more and better analytical aids available to clinical practice, and, in our own research, to use these aids in the further elucidation of clinical problems. These things are self-evident, and I think that no one doubts that this part of the course will be followed. But also we must remember that we are part of a team whose successful operation is essential to the relief of human misery, and that for a team to function well the most cordial relations must be maintained among its members. Further, we must remember that we are the newest members of the team, and as such we can at times and without loss of dignity defer to the team's older members. If we remember these things, I believe it will hasten the day when we too will be counted among the older members of the team.

INTERNATIONAL CONGRESS ON CLINICAL CHEMISTRY

The International Congress on Clinical Chemistry, held September 9–14, 1956, in New York City, lived up to its advance notices and publicity and brought together for the one-week meeting the largest group of clinical chemists ever to assemble in this country.

Almost 700 clinical chemists representing more than 21 countries spent 5 days attending 17 separate scientific sessions and symposia; a very worthwhile technical exhibit, featuring outstanding exhibits of major instrumental and technical advances; scientific exhibits which showed the major researches of key laboratories and institutions; and a round of well-organized social activities that allowed for the more informal and personal meetings among the individual scientists.

The scientific meetings, built around six symposia, featuring twenty invited lectures by both foreign and American scientists, and 119 contributed papers in 17 sessions. Both symposia and contributed papers were featured in CLINICAL CHEMISTRY **2:** 225 (1956) which was devoted to the publication of the abstracts of the Congress.

The Congress also afforded the opportunity to hold complete AACC meetings. The membership meeting had one of the largest attendances of any Association business meeting. The AACC Executive Committee meeting (minutes published in this issue) was able to handle many problems and bring these directly to the membership the following day.

The coverage of the meetings and exhibits by the daily press was outstanding. Major stories concerning the Congress were the feature articles in most columns devoted to science during the Congress week.

The American Association has decided to publish the twenty papers of the six symposia in a special supplement to Volume 3 of this journal. This supplement will be sent free to all subscribers and Association members. Copies will also be available for purchase.

The success of the Congress was due to the hard-working committees appointed by the AACC and the host, the New York Section. Albert E. Sobel, Chairman of the Congress, John G. Reinhold, Congress Secretary, Harry Sobotka, Chairman of the Committee for the Scientific Program, Charles L. Fox, Jr., Chairman for the Scientific and Technical Exhibits, and Harry Goldenberg, Chairman of the Committee on Hospitality and members of all the Congress Committees deserve the tribute of the membership for an outstanding accomplishment.

1956 ERNST BISCHOFF AWARD

Dr. Joseph H. Roe, professor of biochemistry and chairman of the department at the George Washington University Medical School in Washington, D. C., has been selected by the American Association of Clinical Chemists to receive the 1956 Ernst Bischoff Award for his outstanding work in clinical chemistry. The Association presents this award each year to a worker in the field who has distinguished himself by achievement and devotion and has helped solve those chemical problems which arise daily in the practice of the medical arts.

Dr. Roe, a native of Virginia, received his B.A. at Roanoke College and an M.A. at Princeton. He joined the faculty of George Washington University after service in World War I. He received two Ph.D. degrees, one in chemistry from George Washington, the other in physiological chemistry from Yale. He served for ten years as chemist at the university hospital.

Outstanding contributions by Dr. Roe in the three major fields of clinical chemistry are basic research in body metabolism, analytical methods for the clinical laboratory, and teaching. He has published some 80 scientific papers, and has contributed particularly to our knowledge of carbohydrate metabolism, especially of fructose and glycogenesis, and of vitamin C. His name is well known to laboratory workers for his methods for determining calcium (Roe and Kahn), fructose, vitamin C (Roe and Kuether), and amylase (Roe and Smith). He has been an inspiring teacher, commanding the respect and admiration of his students.

FERRIN B. MORELAND
Baylor University
Houston, Texas

PROCEDURE FOR NOMINATION FOR ERNST BISCHOFF AWARDS

The Executive Committee of the American Association of Clinical Chemists has altered the procedure for the selection of the Ernst Bischoff medalist. The award committee shall henceforth consist of a panel of five members to be appointed by the President of the Association. The names of this committee shall not be announced.

All future nominations should be addressed to the National Secretary, Dr. Max M. Friedman, Lebanon Hospital, New York 57, N. Y. Any member of the Association has the privilege of nominating a candidate for the award. The nominee does not have to be a member of the Association nor a resident of the United States, since the award is open to any clinical chemist who has contributed significantly to the specialty.

Five copies of a brief should be submitted for each nomination. The statement of preference should be concise but yet contain pertinent data with reference to the nominee. A bibliography is not required but the areas of contributions should be clearly stated.

The Ernst Bischoff Award consists of a scroll, a medal, and an honorarium of five hundred dollars. It has been presented annually since 1952 through the cooperation of the Ames Company of Elkhart, Indiana. The most recent recipient was Dr. Joseph H. Roe. (See previous item.)

MINUTES OF THE MEETING OF THE AACC EXECUTIVE COMMITTEE

The National Executive Committee of the American Association of Clinical Chemists met in New York City on September 12, 1956. Those present were: ROBERT M. HILL (President), JOSEPH I. ROUTH (Vice-President), LOUIS B. DOTTI (National Treasurer), MAX M. FRIEDMAN (National Secretary), EMMETT B. CARMICHAEL, W. E. CORNATZER, MARTIN RUBIN, JOHN G. REINHOLD (alternate for GEORGE R. KINGSLEY), HARRY SOBOTKA (al-

ternate for OTTO SCHALES), and the following by invitation: Harold D. Appleton, Monroe E. Freeman, Hugh J. McDonald, David Seligson, and Albert E. Sobel.

Carmichael reported for the Committee on Education which had met in San Francisco in the spring of 1955 and in Atlantic City on April 16, 1956. The preliminary recommendations of the committee were discussed at length and the report was sent back to the committee for further study.

Seligson reported for the Committee on Methods and Standards and outlined the future plans. The second volume of *Standard Methods* was nearing completion and its publication may be expected soon. This committee decided that a separate volume for toxicologic methods was not advisable, but that procedures for drugs and poisons would be included in forthcoming volumes. A previous decision was again approved, that the same personnel of the committee be concerned with both standard methods and laboratory standards. (See following item.) It was recommended that funds be sought from appropriate agencies for the purpose of studying the adequacies of commercial standard samples. Approval was given for the sum of $500.00 to be made available to the Committee on Methods and Standards.

Appleton reported for the Board of Editors. The Journal, CLINICAL CHEMISTRY, is carrying on successful operations and there is already a need for further expansion. The Board of Editors requested a supplement of about 450 pages for the purpose of publishing the symposia papers given at the International Congress of Clinical Chemistry. This request was approved. (Since the approval was given, an alternate means of publication of the symposia papers was suggested and is now under consideration by the Executive Committee and the Board of Editors.) The Board of Editors was also instructed to cooperate with *Clinica Chimica Acta*, published by the Elsevier Publishing Co., The Netherlands.

The term of Albert E. Sobel as delegate to the AAAS was extended for three years, and the appointment of Monroe E. Freeman as representative to the International Federation of Clinical Chemistry was extended.

Dotti presented the annual Treasurer's report which follows:

Income

Membership dues	$6,852.50
CLINICAL CHEMISTRY	1,845.65
Standard Methods	115.36
Membership certificates	60.00
Bank interest	147.06
National Science Foundation (for International Congress)	4,000.00
TOTAL	$13,020.57

Expenses

CLINICAL CHEMISTRY Membership, subscriptions and editorial expenses)	$4,739.54
Treasurer disbursement	166.44
Secretary disbursement	1,029.86
Membership refunds	35.40
AAAS meeting	85.60
Section allotments	456.80
Membership committee	56.20
Methods committee	250.00
International Congress	2,257.70
TOTAL	$9,077.54
Balance for year July 1, 1955 to June 30, 1956	3,943.03
Bank balance June 30, 1955	3,755.11
Bank balance June 30, 1956	$7,698.14

In view of the additional costs in publishing a supplement to the journal, and with the need for expanding the size of the present volumes, the Executive Committee approved a recommendation to the membership that the annual dues be increased to $12.50 for members and $9.00 for associate members. (This was later approved at the membership meeting.)

A change in the method of administering the Ernst Bischoff Award was approved. The new procedure will be for a committee of five members to be appointed by the President of the Association and whose names shall not be made public. This committee shall select the recipient, while nominations shall be sent directly to the National Secretary.

The Metropolitan New York Section requested permission to establish an award to be administered by the section. This matter was discussed and it was agreed that any section may establish such awards subject to the approval by the Executive Committee of the Association.

The ethics of contract practice in clinical chemistry laboratories were discussed at some length. The following Resolution was drawn up with legal counsel and approved by the Executive Committee:

Resolution Concerning Unlimited Laboratory Service for an All-inclusive Fee

It is the conviction of the Executive Committee of the American Association of Clinical Chemists that attempts currently being made to provide by contract with a physician unlimited all-inclusive laboratory service for a prearranged single fee without regard to special requirements is not conducive to dependable chemical measurements.

This Committee believes that participation in such plans as they exist is not in keeping with the scientific and professional standards of the American Association of Clinical Chemists.

It strongly urges chemists, both members and nonmembers of the Association, to refrain from participation in such contracts.

Approval was given to discontinue membership certificates due to insufficient demand.

It was the view of the Executive Committee that it should be the aim of the Association that a Certified Clinical Chemist to be in the employ of every hospital. The membership committee was directed to screen the membership for potential diplomates.

Action on legislation as it affects clinical chemistry was deferred to the Committee on Clinical Chemistry of the American Chemical Society, since this committee was actively concerned with the problem.

A motion was made, seconded, and passed that the Stated Annual Meeting of the AACC for 1957 be held within the framework of the American Chemical Society in New York City during September, 1957.

MAX M. FRIEDMAN
National Secretary

RESOLUTION

Whereas, the American Association of Clinical Chemists is concerned

with the accuracy and precision of analytical procedures, and

Whereas, the Association has appointed a Committee on Standards of which David Seligson is Chairman, to investigate the practicability of utilizing a stabilized-standardized serum for the purpose of improving the performance of clinical chemistry, and

Whereas, the Association is now prepared to study and evaluate such a standard serum, be it therefore

Resolved that interested laboratories and organizations be invited to submit such samples for evaluation by the Committee on Standards. The submitting laboratories will be expected to provide funds for an adequate evaluation. The American Association of Clinical Chemists will approve satisfactory products that meet its specifications.

STATED ANNUAL MEETING

The Stated Annual Meeting of the membership was held in New York City on September 13, 1956. More than 100 members were in attendance.

The National Secretary reviewed the transactions of the Executive Committee who had met on the previous day. The first order of business was the recommendation that annual dues be increased to $12.50 for members and $9.00 for associate members, to take effect January 1, 1957. A motion was made, seconded, and passed that dues be set at the above figures.

The Resolution on all-inclusive contracts for laboratory work passed by the Executive Committee was brought to the floor for discussion. It was the consensus of the membership that the Resolution be subject to review and advice by legal counsel. (This was subsequently carried out as noted in the minutes of the Executive Committee.)

A recommendation was made that the Association seek subsidy funds for travel to the International Congress to be held in Stockholm in August, 1957.

The membership was again notified that the Association was carrying on an active employment exchange, and information may be obtained through the office of Dr. Joseph I. Routh, University Hospitals, Iowa City, Iowa.

A recommendation was made to the Executive Committee that annual meetings be held once in three years with the Federation of American Societies for Experimental Biology.

A vote of thanks was given to the Metropolitan New York Section for its part in the success of the meetings of the International Congress of Clinical Chemistry.

DUES FOR 1957

Following the recommendation of the Executive Committee the membership voted on September 13, 1956, that the annual dues be changed to $12.50 for members and $9.00 for associate members. This change of dues is to take effect on January 1, 1957. The annual dues includes a membership subscription to CLINICAL CHEMISTRY as well as a reimbursement of $1.00 for each member to the local sections.

An increase of $1.50 was necessitated by a further expansion of the Journal which in 1957 will include a supplement of about 300 pages for the manuscripts of the invited symposia at the International Congress of Clinical Chemistry held in New York.

LETTER TO THE EDITOR

To the Editor:

Within recent months laboratories in a number of cities have contracted with physicians to provide virtually unlimited laboratory service for any number of patients for a monthly fee. Similar plans have existed in New York City for some time, but it is only recently that attempts have been made to extend them to other parts of the country. The fees charged to the physician range around $60.00 per month. Services such as collection of blood specimens and performance of glucose tolerance and similar tests are included. A few of these laboratories appear to be operated by chemists.

There is much about the provision of unlimited laboratory service for a fixed price contracted for in advance that is potentially harmful. The less scrupulous physician may be tempted to exploit the arrangement by requesting numerous studies not strictly required. Such a physician could, if he chose, collect charges for laboratory work from the patient at the prevailing scale. On the other hand, the laboratory would be under pressure to perform examinations at the least possible expense. Inevitably the quality of the work done would be impaired and the patient's welfare could be jeopardized.

The manner in which these services are being arranged also is most disturbing. Promoters appear to be traveling from city to city and conducting active campaigns of solicitation among physicians. Although provision of laboratory services at lower cost is given as the reason for development of these plans, the prevailing evidence is that financial gain is the actual motive.

The costs of laboratory studies to patients should indeed cause concern to all who are engaged in providing these services. Much might be done to lower costs by organizing such work efficiently. However, attempts to mass-produce analyses introduce factors such as fatigue and boredom, and create new problems of control and supervision that unless successfully met lead to work of poor quality. Moreover, it is likely to be the weak or unsuccessful laboratories that turn to bargain rates, rebates, and similar practices. These would hardly be likely to solve the complicated problems arising in connection with work done on a large scale.

One may ask physicians who sign such contracts whether inexact or erroneous reports will be a bargain. Laboratory directors who resort to actions as dubious as these should realize that they will impair their standing among scientists and reputable physicians.

Clinical chemists who turn to such devices harm their profession and the public they should be serving. It is the opinion of the writer that they should

be denied membership in the American Association of Clinical Chemists.

JOHN G. REINHOLD
Philadelphia, Pa.

SECOND INTERNATIONAL EUROPEAN CONGRESS ON CLINICAL CHEMISTRY

The Second International European Congress on Clinical Chemistry will be arranged by the Swedish Society for Clinical Chemistry in Stockholm, August 19–23, 1957. Introductory lectures will be given on the following topics: Enzymes of Diagnostic Value in Clinical Chemistry, The Influence of Hormones on the Electrolyte Metabolism, Chromatographic Methods and Their Clinical Application, and Clinical Chemistry of the Polysaccharides.

Short papers on these or other subjects in the field of clinical chemistry may be presented by any of the members of the Congress. An exhibition of commercial laboratory instruments will be arranged.

Further information will be given on request by the Congress Bureau. Address Box 12024, Stockholm 12, Sweden.

PUBLICATIONS IN *THE CLINICAL CHEMIST*

The following is a list of papers that appeared in *The Clinical Chemist*, newsletter of the Association during the years 1949–54. This publication was terminated with the appearance of CLINICAL CHEMISTRY in February, 1955.

An Extrapolation into the Future of Clinical Microchemical Analysis: Glick, D., Vol. 4, No. 5; *Chem. Abstr.* **47,** 4782g. *Chem. Eng. News* **31,** 139 (1953). Ernst Bischoff Award Lecture.

Electromigration in Stabilized Electrolytes, Part I. The Development of the Technique: McDonald, H. J., Marbach, E. P., and Urbin, M. C., Vol. 5, No. 2; *Chem. Abstr.* **47,** 9726g.

Electromigration in Stabilized Electrolytes, Part II. Factors Influencing Mobility: McDonald, H. J., Lappe, R. J., Marbach, E. P., Spitzer, R. H., and Urbin, M. C., Vol. 5, No. 3; *Chem. Abstr.* **48,** 7400b.

Electromigration in Stabilized Electrolytes, Part III. Applications of the Techniques: McDonald, H. J., Lappe, R. J., Marbach, E. P., Spitzer, R. H., and Urbin, M. C., Vol. 5, No. 4; *Chem. Abstr.* **48,** 7400b.

A Standardized Plasma Preparation Suitable for Use as a Control in the Determination of Prothrombin Time: Hodes, M. E., Vol. 5, No. 4.

Diabetogenic Effects of Insulin—Hypoglycemia: Somogyi, M., Vol. 5, No. 5; *Chem. Abstr.* **48,** 6599g. Ernst Bischoff Award Lecture.

Chemical Evaluation of the Functions of the Liver, Part I. Reinhold, J. G., Vol. 5, No. 6; *Chem. Abstr.* **48,** 5349e.

A Note on Quantitative Urobilinogen Determinations: Balikov, Bernard, Vol. 5, No. 6.

Chemical Evaluation of the Functions of the Liver, Part II. Reinhold, J. G., Vol. 6, No. 1; *Chem. Abstr.* **48,** 13013h.

A Stable Somogyi Substrate for the Determination of Serum Amylase: Wenger, R. G., Vol. 6, No. 1.

Chemical Evaluation of the Functions of the Liver, Part III. Reinhold, J. G., Vol. 6, No. 2; *Chem. Abstr.* **48,** 13013h.

Pontacyl Standards for the Biuret Method for Total Serum Protein: Sanshuk, D., and Freeman, M. E., Vol. 6, No. 2.

The Determination of Erythrocyte Sedimentation Rate with Blood Specimens Collected for Prothrombin Time Estimations: Weiner, M., and Simson, G., Vol. 6, No. 3.

Preparation of a Stable Solution of Pooled Human Serum: Maher, J. R., Vol. 6, No. 3.

Polarography and Its Applications to Clinical Problems: Pecsok, R. L., Vol. 6, No. 3.

Lipoproteins: Mehl, J. W., Vol. 6, No. 4.

ABSTRACTS

Editor: ELLENMAE VIERGIVER. *Contributors:* JOSEPH S. ANNINO, GLADYS J. DOWNEY, CLYDE A. DUBBS, ALEX KAPLAN, MARGARET M. KASER, MIRIAM REINER, HERBERT THOMPSON

Improved substrate for study of clearing factor. L. Zuckerman and H. O. Singher (*Ortho Research Foundation, Raritan, N. J.*)

A substrate was prepared by diluting 50% coconut oil emulsion (Ediol, Schenley Laboratories, Inc.) 1:100 with normal plasma or albumin. Normal citrated dog plasma was obtained from fasting animals. The plasma was used the same day or was frozen until needed. Heparinized plasma was obtained by drawing blood 10 minutes after the intravenous injection of 50 μg. of heparin per kg. of body weight. Bovine albumin (22% solution, Ortho Pharmaceutical Corp.) was diluted to 3% concentration with 0.32% trisodium citrate. Phosphate buffer (*p*H 7.2, ionic strength 0.1) diluted with an equal volume of physiologic saline was used to adjust volumes in the tubes of the test mixtures. Into a Lumetron micro tube were pipetted 0.1 ml. of the 0.5% oil emulsion plus 0.5 ml. of 3% albumin or 0.5 ml. of normal plasma. This mixture, heparinized plasma, and phosphate buffer were warmed in separate tubes to 37.5°. Then 0.4 ml. of heparinized plasma or heparinized plasma diluted with buffer was added to the substrate. Turbidity readings were made immediately and at 5- or 10-minute intervals with a Lumetron photoelectric colorimeter at 660 mμ against a water blank. Results were expressed as increases in light transmission. With the improved substrate and the relative amounts of substrate, protein, and heparinized plasma indicated, the procedure is reproducible and the clearing reaction can be expressed linearly.—*Proc. Soc. Exp. Biol. Med.* **89,** 315 (1955). (M. M. K.)

A colorimetric method for the estimation of serum magnesium. A. J. Smith (*King's College, Newcastle-upon-Tyne, England*).

Calcium in serum is removed by precipitation as the oxalate. After removing the serum proteins by precipitation, the Mg is determined by adding a dilute solution of eriochrome black T at *p*H 10.1. The intensity of the Mg-eriochrome black T complex is measured at 520 mμ.—*Biochem. J.* **60,** 522 (1955). (A. K.)

The amount of trapped plasma in the red cell mass of the hematocrit tube. F. G. Ebaugh, Jr., P. Levine, and C. P. Emerson (*Massachusetts Memorial Hospitals and Boston University School of Medicine, Boston, Mass.*).

The cell volume in 52 blood samples with hematocrit readings ranging from 18.7 to 75.4% has been measured directly by hematocrit readings and indirectly computed from the dilution of chromium^{51}-labeled hemoglobin solution. The

difference between these values is considered to represent the volume of plasma "trapped" in the packed cell mass of centrifuged samples. The data demonstrate a positive correlation between the quantity of plasma trapped and the height of the red cell column. The amount of trapped plasma was 0.8% or less for a hematocrit reading of 40%, 2% for a hematocrit of 50%, and 4% for a hematocrit of 68%.—*J. Lab. Clin. Med.* **46,** 409 (1955). (E. V.)

Lactic dehydrogenase activity in blood. F. Wroblewski and J. S. LaDue (*Sloan-Kettering Institute, New York, N. Y.*).

The following method was developed for the determination of lactic dehydrogenase (LD) in serum: to 2.4 ml. of *p*H 7.4 phosphate buffer add 0.1 ml. of serum and 0.1 ml. of reduced diphosphonucleotide (DPNH, 2.5 mg./ml.). After 20 minutes add 0.1 ml. of sodium pyruvate (2.5 mg./ml.). The optical density at 340 mμ is followed for 3 to 5 minutes with the Beckman Model DU spectrophotometer. Activity is expressed as units per ml. of serum, one unit being equal to a decrease in optical density of 0.001 per minute per ml. The venous serum activity of 161 normal human adults varied from 260 to 850 units, with a mean value of 470 units. Values for whole blood hemolysates ranged from 16,000 to 67,000 units, with a mean of 34,000 units. LD activity was studied in experimental animals and in human subjects. Like serum glutamic oxaloacetic transaminase, lactic dehydrogenase was increased in serum following myocardial infarction. It was also increased in diabetic acidosis, acute stem cell leukemia, chronic myelogenous leukemia, and hepatitis.—*Proc. Soc. Exptl. Biol. Med.* **90,** 210 (1955). (M. M. K.)

New olive oil emulsion for lipase and new observations concerning "serum lipase." H. Tauber (*University of North Carolina, Chapel Hill, N. C.*).

A stable olive oil emulsion was prepared with the aid of "Carbopol 934," a new hydrophilic colloid with carboxyl groups which disperses in water and forms a stable gel upon neutralization. One gram of Carbopol 934 and 200 ml. of water are mixed in a Waring blendor followed by the addition of 5 gm. of acacia in 100 ml. of water with continued mixing. Finally, 100 ml. of olive oil are poured in, and sodium hydroxide is added until *p*H 7.00 is obtained. Two milliliters of toluene are added and homogenization is continued 15 minutes. Air is removed from the emulsion in a desiccator with the aid of vacuum. The emulsion keeps in the refrigerator for several weeks without separating and without appreciable increase in its titration blank.

To determine lipase activity 1 ml. of enzyme solution, 5 ml. of olive oil emulsion, and 0.5 ml. of phosphate buffer of *p*H 6.56 are incubated for 20 hours at 37°. For the controls the enzyme solution is added at the end of the incubation period. Three milliliters of 95% alcohol are added and the digests are titrated with 0.05N NaOH using phenolphthalein as indicator. The results are expressed as the number of milliliters of 0.05N NaOH required for neutralization. Hy-

drolysis is fairly linear for 48 hours, but activity is not proportional to increasing quantities of rabbit serum. Serum lipase appears to lose none of its activity in 24 hours at 37°. It is destroyed by heating 30 minutes at 56°, but serum so treated can be shown to contain an antilipase. Hemoglobin was shown to be a powerful lipase inhibitor.—*Proc. Soc. Exptl. Biol. Med.* **90,** 375 (1955).

(M. M. K.)

Studies of serum mucoprotein (seromucoid). I. A turbidimetric method. J. de la Huerga, A. Dubin, D. S. Kushner, H. A. Dyniewicz, and H. Popper (*Northwestern Medical School, Chicago, Ill.*).

A simple turbidimetric method for the determination of serum mucoprotein is described. To 2 ml. of serum add 18 ml. of 0.85% NaCl. Mix and add dropwise 10 ml. of 1.8N perchloric acid solution. After standing 15 minutes at room temperature, filter through Whatman # 50 paper. Transfer 15 ml. of filtrate to an 18- x 50-mm. test tube calibrated at 6 ml. Add 3 ml. of 5% phosphotungstic reagent (5% phosphotungstic acid in 2N HCl) and mix by inverting 3 times. After 20 minutes, centrifuge 5 minutes at 500 rpm. Siphon off the supernatant fluid to the 6-ml. mark and discard this portion. Invert the tube 3 times and transfer the contents to a cuvet. Read against a water blank at 650 mμ. The instrument used in this study was the Coleman Universal Spectrophotometer. Armour's bovine serum albumin similarly treated and used as a standard emeonstrated a linear relationship between concentration and optical density. The normal range for 70 normal adults was 55 to 105 mg./100 ml., with a mean of 77 mg.—*J. Lab. Clin. Med.* **47,** 403 (1956). (G. J. D.)

The occurrence and determination of delta-aminolevulinic acid and porphobilinogen in urine. D. Mauzerall and S. Granick (*Rockefeller Institute, New York, N. Y.*).

Methods are described for the determination of Δ-aminolevulinic acid (Δ-AL) and porphobilinogen (PBG), both of which are intermediates in the biosynthesis of porphyrins. When a urine sample is passed through an iron-exchange column containing Dowex 2, PBG is retained. The PBG is then eluted and its concentration determined colorimetrically with Ehrlich's reagent. When the washings from the first column are passed through Dowex 50, Δ-AL is retained. The Δ-AL is then eluted, allowed to react with acetylacetone, and the resulting pyrrole is determined colorimetrically with modified Ehrlich's reagent.—*J. Biol. Chem.* **219,** 435 (1956). (J. S. A.)

Blood pyruvic acid, serum gamma globulin, and other tests of hepatic function. R. K. Parida and R. M. Kark (*Presbyterian and Cook County Hospitals, Chicago, Ill.*).

The concentration of blood pyruvic acid levels is of limited value in assessing hepatic dysfunction in man. However, in both health and in disease a high degree

of positive correlation exists between the concentration of serum gamma globulin (and thymol turbidity) and the basal blood pyruvate. There is a negative correlation between serum cholinesterase activity and pyruvate concentrations.—*J. Lab. Clin. Med.* **47**, 42 (1956). (G. J. D.)

Ultramicro determination of acetylcholine in cerebrospinal fluid. R. P. MacDonald, C. Gerber, and A. Nielson (*Harper Hospital, Detroit, Mich.*).

The concentration of acetylcholine in cerebrospinal fluid which is of interest in many neurologic disturbances is determined as follows: label three tubes *A* (unknown), *B* (blank), and *C* (standard). To tube *A* add 100 λ of 1N HCl and 100 λ of prostigmin bromide (10 mg. in 4.4 ml. of water). To all 3 tubes add 1 ml. cerebrospinal fluid and let stand 10 minutes at room temperature. Add 100 λ prostigmin bromide and 100 λ of 1N HCl to tubes *B* and *C*. To tube *C* also add 100 λ of standard acetylcholine chloride (10 mg. dissolved in 100 ml. of water). Transfer 250 λ of each of the 3 tubes to clean tubes. Add 50 λ of alkaline hydroxylamine solution (1 part of 2M hydroxylamine hydrochloride in water, combined with 2 parts of 3.5N NaOH just before using) to each tube and mix thoroughly. Add 25 λ of 4N HCl, mix and let stand 1 minute. Add 25λ ferric chloride solution (0.37M in 0.1N HCl) to each tube, mix thoroughly, and let stand for 10 minutes. Transfer to microcuvets and read the optical density at 540 mμ. This procedure is simple and rapid and control studies indicate an accuracy well within the limits required for clinical determinations. —*Am. J. Clin. Pathol.* **25**, 1367 (1955). (H. E. T.)

A method for protein-bound iodine: The kinetics and the use of controls in the ashing technique. H. L. Thompson, M. R. Klugerman, and J. Truemper (*Second Army Area Medical Laboratory, Fort George G. Meade, Md.*).

A modification of Barker's dry-ashing method for PBI is presented. The effects of variable ionic constituents on the catalytic system and methods of control to ensure maximum accuracy and reproducibility are outlined. Data are given showing the extent of adherence of ceric ion to Beer's law for several instruments, namely, the Coleman 6 and 6A, the Evelyn, the Klett-Summerson, and the Beckman DU.—*J. Lab. Clin. Med.* **47**, 149 (1956). (G. J. D.)

Electrophoresis of cationic proteins on filter paper. K. H. Monty, M. Morrison, E. Alling, and E. Stotz (*University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*).

A method is proposed for overcoming adsorption of cationic proteins by filter paper during paper electrophoresis. The paper is treated with a 1% solution of the detergent Octab prior to electrophoresis. A method for subsequent electrophoresis and staining is described and comparative patterns are shown. The method is designed to minimize "trailing" during electrophoresis.—*J. Biol. Chem.* **220**, 295 (1956). (J. S. A.)

A new method for the anaerobic separation of plasma. J. T. Murray (*Christchurch Hospital, Christchurch, New Zealand*).

An apparatus and procedure for the anaerobic separation of plasma for acid-base balance and electrolyte studies which obviates the use of paraffin oil is described.—*Am. J. Clin. Pathol.* **26,** 90 (1956). (H. E. T.)

Determination of blood pH and a new cell for blood pH. J. T. Murray (*Christchurch Hospital, Christchurch, New Zealand*).

Details for the construction and use of a special cell for the determination of *p*H on 0.5 to 0.6 ml. of blood or plasma under anerobic conditions are described.—*Am. J. Clin. Pathol.* **26,** 83 (1956). (H. E. T.)

Stability of protein, enzyme and nonprotein constituents of stored frozen plasma: Use in standardization and control of chemical procedures. R. L. Walfor, M. Sowa, and D. Daley (*University of California School of Medicine, Los Angeles, Calif.*).

Analysis of frozen plasma stored for 6 months showed that the concentration of total protein, urea, uric acid, creatinine, chloride, and amylase were within 3 per cent of the initial values. Nonprotein nitrogen, alkaline, and acid phosphatase were 11, 25, and 28 per cent, respectively, less than the initial values, and glucose was 5.4 per cent less.—*Am. J. Clin. Pathol.* **26,** 376 (1956). (H. E. T.)

An improved method for determination of total serum cholesterol. D. M. Colman and A. F. McPhee (*University of California, Berkeley, Calif.*).

A method for the determination of total cholesterol in 0.1 ml. of serum is presented. It has been shown that a quantitative estimation of cholesterol can be made in the presence of protein degradation products. This means that the entire procedure can be carried out in one vessel except for a nonquantitative transfer to a colorimeter tube. The serum is saponified with KOH, extracted with acetone-alcohol, and the cholesterol precipitated with digitonin. The color development is a modification of the usual Liebermann-Burchard procedure. Accuracy and precision have been shown to be of a high order.—*Am. J. Clin. Pathol.* **26,** 181 (1956). (H. E. T.)

Index to Volume 2

* Abstract of paper delivered at The International Congress of Clinical Chemistry, 1956.

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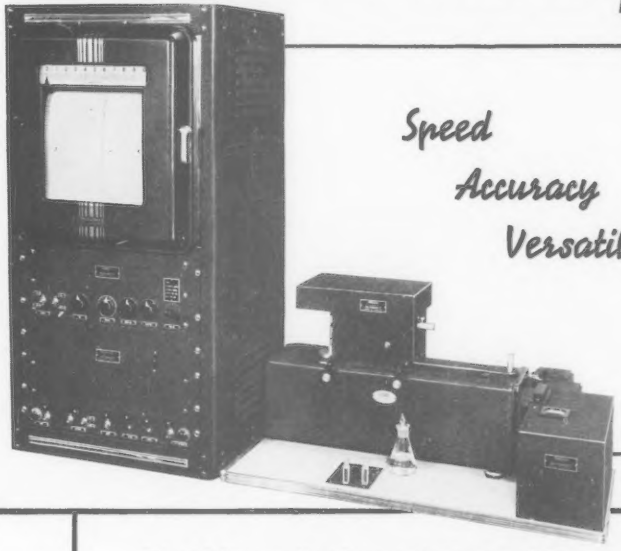

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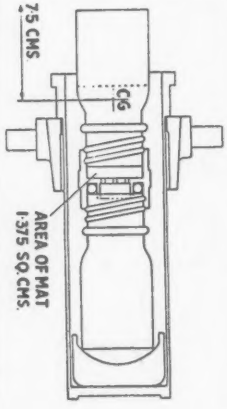

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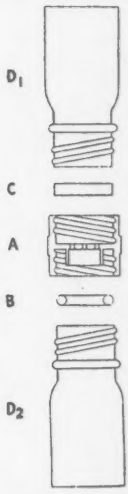

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